

EVALUATION AND PROPAGATION OF CHINESE WINGNUT, *PTEROCARYA STENOPTERA*, AS A NEW  
BIOENERGY FEEDSTOCK

BY

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THESIS

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### ABSTRACT

Short rotation woody crops (SRWC) as a source of biomass for energy production could play a vital role in solving the problems associated with climate change, global warming, and the rising price and diminishing supplies of carbon-based fossil fuels. In order to meet these challenges, new species will need to be assessed for use as SRWCs. Chinese wingnut, *Pterocarya stenoptera*, has been reported to exhibit several characteristics such as fast growth rate and suitability to a wide range of soil and moisture conditions that could make it attractive as a potential source of woody biomass. Due to lack of past work with the species, little is known regarding clonal and seed propagation, coppicing ability, biomass composition and growth rate. In order to determine the best methods of propagation for this species, hardwood and semi-hardwood cuttings were treated with a range of Indole-3-butyric acid (IBA) concentrations suspended in a mixture of 25% ethanol or suspended in talcum powder. The cuttings were subjected to wounding or non-wounding treatments and treated with liquid quick dip IBA concentrations of 1000, 2500 and 5000 ppm or mixed with talcum powder to produce concentrations of 1000, 3000, and 8000 ppm IBA. The cuttings were grown under mist conditions for 6 to 9 weeks with 14 hour photoperiods. Results were analyzed using analysis of deviance in SAS. The number of roots per cutting and rooting percentages varied with IBA concentrations but results also revealed a high degree of variability in the mist bench conditions. The results of this study indicate that the preferred method of propagation of *Pterocarya stenoptera* by cuttings would be to utilize wounded, semi-hardwood, subterminal cuttings treated with either liquid dip or talc powder quick dip with IBA levels ranging from 2500 to 8000 ppm under mist conditions.

In order to better understand propagation of Chinese wingnut from seed, a replicated study was conducted with batches of seeds being removed from stratification at 4.5 °C in moist heat sterilized sand every 5 days for a period of 60 days. Seeds were then planted in greenhouse trays and germinated under greenhouse conditions. A logistic regression model was fit to assess the impact of stratification period on seed germination. The probability of seed germination increased with increasing days of stratification up to 30 days, with only minor changes after 30 days, indicating an optimal stratification period of 30 days.

In order to better understand the coppicing ability of Chinese wingnut, a study was designed to test the effect of different cutting heights on regrowth of shoots from the main stem. The study was conducted using nine-month-old containerized wingnuts grown from seed under greenhouse conditions. Treatments consisted of removing all leaves from the plant and cutting the main stem so that 0, 5, 10, or 15 buds remained with treatments having average heights of 4.6 ( $\pm$  2.5), 8.0 ( $\pm$  1.7), 11.9

( $\pm 2.5$ ), and 15.3 ( $\pm 2.3$ ) cm, respectively. Data was collected on the number of shoots and leaves, and total leaf dry mass per plant after 33 days. Dry mass was analyzed via Analysis of Variance (ANOVA) and shoot and leaf count data were analyzed via Analysis of Deviance. Dry mass was affected ( $\alpha = 0.001$ ) by the number of buds left remaining on the stem, with the 15-bud treatment having a mean dry regrowth shoot mass of 4.37g. The number of shoots regenerated from the three treatments was significant ( $\alpha = 0.001$ ) with the 15-bud treatment producing the greatest number of shoots (10.3/plant); however, the number of leaves produced by treatments was not significant, suggesting that plants with fewer shoots had a greater density of canopy. Results suggest that a harvest system should allow some degree of above ground biomass to allow for fast coppice regrowth.

When considering the suitability of a woody plant for use as a biofuel feedstock an accurate picture of the chemical composition of the biomass is necessary. To that end compositional analysis was conducted on *Pterocarya stenoptera* biomass. Biomass samples were collected at the University of Illinois bioenergy research farm and from locations in seven Midwestern states. The samples were collected from tree limbs during winter dormancy with samples being divided by age class to contain the most recent growing years 1-4. Samples were oven dried and ball milled. High pressure liquid chromatography analysis was used to determine structural carbohydrates such as lignin, hemicellulose, monosaccharides, and acetate content. High temperature incubation was used to determine Klason lignin content, and extractives content was determined using an ethanol and water extraction. Test procedures followed those set forth by the National Renewable Energy Laboratory. Samples collected from the fourth year of growth had the following compositional profile: cellulose  $26.9 \pm 2.37\%$ , hemicellulose  $16.3 \pm 0.98\%$ , lignin  $24.6 \pm 0.79\%$ , ash  $1.99 \pm 0.52\%$ , extractives  $12.8 \pm 2.32\%$  and acetyl  $3.12 \pm 0.18\%$ . Results indicate comparatively low levels of cellulose at  $26.9 \pm 2.37\%$  for 4 year old wood compared to common biomass production species such as *Robinia pseudoacacia*, *Populus deltoides*, and *Salix alba* that have cellulose values ranging from 40 - 42%. The low levels of cellulose found in the compositional analysis studies reveal that Chinese wingnut, *Pterocarya stenoptera*, is not an ideal candidate for biomass production.

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# I ntroduction

This paper examines propagation methods, biomass composition and coppicing ability of Chinese wingnut, *Pterocarya stenoptera*, as related to its potential as a feedstock for biofuel production.

*Pterocarya stenoptera*, commonly known as Chinese wingnut, is native to China, Korea and Taiwan and is now naturalized in the United States, Europe and many other locations. Chinese wingnut is deciduous and 40-70' tall with branches that spread as wide as the tree is tall. It has compound leaves 6-12" long composed of 11-21 finely toothed oval leaflets. Seed clusters are 6-12" long with green strings of winged seeds suspended below its branches. The seeds turn brown before seed drop in the fall. Specific gravity ranges from 0.32 g/cm<sup>3</sup> at the pith to 0.439 g/cm<sup>3</sup> in stem material (Minghong, Zou, et al. 2002). Chinese wingnut has been utilized to a limited degree as a shade tree in the southern United States (Dirr, 1998; Gilman and Watson, 1994).

As worldwide demand for energy increases and supplies of fossil fuels become scarce, alternative fuels will become increasingly important. Energy derived from biomass feedstocks grown in the United States will not only help to reduce the use of fossil fuel but will also help create energy security, lower carbon emissions and provide an avenue for rural economic growth (Perlack and Stokes, 2011). Biomass is utilized around the globe in solid, liquid and gaseous states to supply heat, liquid fuel and energy (Bridgwater, 1999). Biomass is the world's 4th largest supply of energy after coal, petroleum and natural gas, currently accounting for 10-14% of the world's energy production (Putun et al., 2001). Most projections of global energy use predict that biomass will account for around 10-45% of primary energy in the coming decades (Keoleian and Volk, 2005). Unlike traditional fossil fuels, biomass is both renewable and closer to being carbon neutral (Chang, 2007). Biomass has the ability to store solar energy in the form of chemical bonds within the plant material. Perennial biomass such as woody material has the unique advantage of storing energy from multiple seasons of growth in the field until the time of harvest. This storage occurs in both above ground harvestable biomass and long-term underground storage as an environmental benefit.

Although the cultivation of closely spaced fast growing trees has been around since antiquity, the development of short rotation woody crops (SRWC) for fuel and fiber began in the early 20th century with the development of tree breeding programs (Dickman, 2006). Short rotation woody crops

gained further attention in the 1960's with the introduction of the "silage sycamore" concept during the OPEC oil embargo of 1973, leading to statistically designed trials in the United States and elsewhere (Dickman, 2006). Purpose grown trees produced as SRWC offer several advantages over other biomass crops such as annuals or perennials needing yearly harvest. Many of the benefits of purpose grown woody crops are derived from the ability to be harvested at any time during the year and the ability to coppice and regrow (Sims, 2004). The flexibility of harvest time allows for lower volumes of material needing to be stored on site at a processing facility, leading to lower storage and handling costs. The need for less storage also lowers degradation and shrinkage associated with long term storage of biomass. The ability to harvest at any point during the year can also help to minimize risk associated with annual yield fluctuations. Multiple end uses of woody biomass such as traditional forest products and power generation via direct combustion or co-firing with coal, alleviate risk when transitioning or starting a woody biomass plantation (Hinchee et al., 2009). Multiyear rotations minimize environmental impacts associated with annual biomass harvest by allowing for greater durations between harvests with minimal disturbance to the land (Hinchee et al., 2009).

Given that SRWC are less resource intensive and can be grown on less productive or marginal lands, the large scale adoption of SRWC for production of bioenergy would create an economically and environmentally sustainable use for land that is currently underutilized (Perlack and Stokes, 2011). The production of SRWC grown on land that is in current set aside programs, such as the Conservation Reserve Program (CRP), along with producing biomass on underutilized land, has been suggested as a path towards phasing out agricultural subsidies (Hall et al., 1993).

Cost effective sexual and asexual propagation is necessary for any crop being considered for improvement. Given the expense and labor associated with other more costly forms of asexual or clonal propagation such as tissue culture propagation, it is important to determine if propagation from cuttings treated with Indole-3-butyric acid is possible. The first objective of this study was to further the understanding of seed stratification, and cloning of Chinese wingnut from cuttings of hardwood and semi-hardwood material collected from terminal and subterminal stem segments and treated with varying levels of IBA in both liquid and quick dip form.

Many biomass characteristics are important in the evaluation of a woody species for potential use as a short rotation woody crop for the production of biomass for bioenergy. The conversion of biomass into biofuel is complicated, involving many steps and variables. In order for a biomass crop to be suitable for the cost effective conversion to biofuel it must possess characteristics that allow for easy pretreatment, fractionation of carbohydrates and the conversion of carbohydrates to fuel. The second

objective of this study is to determine the biomass composition of Chinese wingnut to determine suitability for use as a biomass crop in the production of biofuel.

# Literature review

## Clonal propagation

An important tool in the development of any biomass crop is the ability to easily propagate the crop by both sexual and asexual means. Sexual propagation can be useful in the seeding of crops and the preservation of genetic diversity in the crop. Asexual propagation becomes important in crop improvement efforts whereby desirable characteristics can be preserved and incorporated into future crops.

Vegetative propagation serves as the primary method for clonal propagation of plant materials in, forestry, plantation agroforestry and many sectors of the horticulture industry. Vegetative cloning allows for multiplication of the same genotype, which is important for dissemination of desirable traits (Hartman and Kester, 2011). In the past, much has been accomplished with the cloning of eucalyptus (*Eucalyptus* sp.), poplars (*Populus* sp.) and willows (*Salix* sp.) (Ritchie, 1997). Large scale eucalyptus programs began in the 1950's in the People's Republic of Congo, where currently 1.2 million cuttings are set annually (Ritchie 1997). Poplar and willow have been successfully cloned for hundreds of years in Europe, mid-Asia and the near-East (Ritchie, 1997).

In some cases the formation of adventitious roots on cuttings requires no more special care than placing the cutting into moist earth, however for difficult to root species application of root inducing chemicals or hormones is necessary. It has been widely demonstrated and reported that auxins play a critical role in adventitious root formation; however, the processes by which auxin stimulates adventitious root development is poorly understood. Auxin is necessary for the initiation of adventitious root tissue with the division of the first root initial cells being dependent on endogenous or exogenously applied auxin (Haissig, 1972). Auxins along with gibberellins and cytokinins are considered to be growth promoting hormones (Hartman and Kester, 2011). Of the five major growth and inhibitory hormones, only auxin and cytokinins are required for plant viability, (Taiz and Zeiger, 2010; MacAdam, 2009). To date, no plant or mutant has been found lacking auxin and cytokinins, suggesting that while other hormones and promoters function as "on or off switches" for various functions, auxin and cytokinins are required at varying levels constantly (Taiz and Zeiger, 2010).



Auxins are a group of tryptophan-derived signals that are responsible for many aspects of plant development (Woodward and Bartle, 2005). Auxins contribute to controlling growth and development, early stages of embryogenesis, apical dominance and root formation (Went and Thimann, 1937). Plants may contain more than one auxin but the most prevalent form is that of indole-3-acetic acid (IAA) (MacAdam, 2009).

Research in the application of auxin to stimulate root growth began after the discovery of auxin's role as a plant growth regulator in the 1930's. Thimann and Koepli (1935) reported the synthetic preparation of indole-3-acetic acid (IAA), a naturally occurring auxin known to have root promoting properties, and demonstrated its practical use in stimulating root growth in cuttings. Not long after the discovery of IAA, the synthetic auxins indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) were shown to be more effective in stimulating root growth (Zimmerman and Wilcoxon, 1935). The greater ability of IBA to promote rooting as compared to IAA is attributed to the increased stability of IBA both in the plant and in solution (Nordström et al., 1991). It was not until 1989 that it was determined that IBA naturally existed in plants at lower levels as a conversion product of IAA (Epstein et al., 1989; Ludwig-Müller and Epstein, 1994).

It is generally accepted that IBA is more effective than IAA or other forms of auxin at inducing rooting. Zolman et al. (2000) showed that IBA efficiently induces lateral rooting at concentrations that do not inhibit root elongation. Genetic studies in *Arabidopsis* and bioassays in a variety of plants show that IBA induces rooting by conversion to IAA in a process resembling peroxisomal fatty acid  $\beta$ -oxidation (Bartel et al., 2001); however it has been proposed that IBA may function without conversion to IAA (Ludwig-Müller, 2000; Poupart and Waddell, 2000).

Auxin is produced in young leaves and transported via active polar transport and through the phloem where at the site of activation it influences a host of plant functions and behavior (Pop et al., 2011). Although it is commonly accepted that plants utilize several pathways to synthesize auxin, none of the pathways has yet been fully defined (Woodward and Bartel, 2005). Chemical and genetic studies have shown that transport of auxin from the site of origin to distant sites is necessary for plant growth and development. For example, auxin transport is necessary for proper root development (Bhalerao et al., 2002) and vascular tissue development (Mattsson et al., 1999).

Early work with oat coleoptile curvature tests, illustrating the nature of polar transport, have determined that auxin moves mainly in a basipetal fashion from the apical to basal ends of excised coleoptiles. Polar transport of auxin proceeds in a cell to cell fashion where auxin exits the cell through the plasma membrane, diffuses across the compound middle lamella and enters the cell below via the

plasma membrane. This process operates independent of gravity and requires metabolic energy as has been proven by the hindrance of polar transport by O<sub>2</sub> deprivation and metabolic inhibitors (Taiz and Zeiger, 2010). Recently however, it has been demonstrated that the phloem can account for a significant amount of auxin transport; more specifically, that the phloem is responsible for acropetal transport from interior regions of the root structure out to root tips (Taiz and Zeiger, 2010).

Biosynthesis of IAA is associated with rapidly dividing tissues, most commonly developing shoots (Ljung et al., 2002). Shoot apical meristems, young leaves, developing fruit, and seeds are the primary sites of IAA synthesis; however, the majority of plant tissues are capable of synthesizing IAA (Ljung et al., 2002). During development of Arabidopsis, auxin concentrations are initially highest in the young leaf primordia, then during development, auxin production shifts basipetally along the margins and later in the central region of the lamina. This shift in auxin production correlates closely with the maturation of leaf development and vascular differentiation (Aloni, 2001).

IAA is structurally related to the amino acid tryptophan, and early work with auxin focused on tryptophan as a precursor to auxin (Normanly, 2010). Moreover, a large body of evidence has also shown that IAA can be synthesized from precursors of tryptophan (Woodward and Bartel, 2005). Multiple pathways exist for the synthesis of IAA; however, the pathways can be broken down into two main categories: tryptophan dependent, if IAA is a product of metabolism of tryptophan, or as tryptophan independent, if IAA is derived from an early indolic precursor of tryptophan (Normanly, 2010). The majority of IAA production occurs via the tryptophan dependent pathway (Normanly, 2010). For the purposes of brevity, this paper will not explore synthesis and degradation of IAA, however if the reader wishes to know more, Normanly (2010) provides a comprehensive review of auxin biosynthesis and metabolism.

Adventitious root formation is the primary mechanism of vegetative propagation (Hartman and Kester, 2011). Adventitious roots are postembryonic roots that can form on stem tissue under stressful conditions (Geiss et al., 2009; Li et al., 2006). The development of adventitious roots in woody plant propagation has been related to the presence of auxin, with physiological stages of rooting correlated to endogenous levels of auxin (Heloir et al., 1996). Application of an exogenous auxin, along with wounding, serves to increase endogenous auxin synthesis and is thought to play a major role in the process of dedifferentiation leading to adventitious root formation (Pop et al., 2011). Work with rice mutants that improperly express the PIN-FORMED1 (OsPIN1) gene that is thought to be involved in polar transport of auxin, shows that non normal levels of auxin distribution and transport affect adventitious

root formation and other processes, illustrating that auxin distribution and concentration are leading factors in adventitious root formation (Xu et al., 2005)

Research by Ludwig-Muller et al. (2005) using stem segments of *Arabidopsis* has shown that IAA and IBA play different roles in the regulation of adventitious root formation. Applications of TIBA, an auxin polar transport inhibitor, to the hypo-cotyledons, lowered the rate of root formation, indicating that exogenous IBA and endogenous IAA interact to promote adventitious root formation in *Arabidopsis* (Fabijan et al., 1981). For most difficult-to-root species it is commonly accepted that IBA is more effective at stimulating adventitious rooting than IAA (Hartman and Kester, 2011). The reasons for this may be: higher stability, differences in metabolism, differences in transport, and that IBA may act as a slow release source for IAA (Ludwig-Muller et al., 2005).

In addition to auxin, ethylene has also been discovered to be a root promoter by Pan et al. (2002) who demonstrated that IAA induces ethylene production and the increased ethylene possibly acts as a stimulus for adventitious rooting. This correlates with the finding that auxin induces the AA synthase4 gene which in turn produces an enzyme that catalyzes the first step in ethylene biosynthesis in higher plants (Abel et al., 1995). Work with a number of isolated mutants of *Arabidopsis* that have resistance to both hormones has demonstrated the relationship of auxin and ethylene (Muller et al., 1998). Work with mutants of *Arabidopsis* and tomato (Negi et al., 2008; Negi et al., 2010) showed that ethylene is an important regulator of the developmental process in higher plants and that several of the physiological processes involved in adventitious rooting involve complex interactions of auxin and ethylene (Sukumar, 2010). Aside from increased ethylene production, auxin is also responsible for rapid initial cell growth responses that may involve auxin induced changes in pH, calcium levels and gene expression (Pop et al., 2011).

### **Root formation**

The formation of new roots can arise from two distinct processes, latent root initials and wound-induced roots. Latent root initials, otherwise known as preformed root initials, consist of locations on the stem where plant cells are pre-dedifferentiated to allow for root formation, with many easily rooted genera such as *Salix*, *Populus* and *Citris medica* possessing latent root initials (Lovell and White, 1986); whereas wound-induced roots are needed for rooting of most plant species without latent root initials (Cline and Neely, 1983).

Wound induced roots are formed when living cells are injured by cutting, thus initiating a response to wounding (Cline and Neely, 1983). The response to wounding has three distinct phases. Initially cells that have been injured in the wounding die, forming a necrotic plate. Suberin also seals the

wound to form a plate that protects from pathogens and desiccation. After the initial response to wounding, the cells behind the newly formed plate begin to divide to form a layer of parenchyma cells which in turn gives rise to a wound periderm. And third, cells within immediate proximity of the cambium and phloem form the nascent stages of *de novo* adventitious roots (Hartman and Kester, 2011).

The formation of adventitious roots arising from *de novo* root primordia created in a wounding response consists of four stages, dedifferentiation or remeristematic, initiation as cells begin to divide and form organized groups of root initials, differentiation of root primordia, and elongation (Davies et al., 1982). In most cases, adventitious roots originate from young parenchyma cells in secondary phloem, however it has also been reported that rooting can initiate in vascular rays, cambium, phloem, callus and lenticels (Ginzburg, 1967; Harbage et al., 1993). Regardless of the location where root tissue is first initiated, the fully developed root primordia forms in the phloem and cortex (Hartman, Kester 2011). At emergence, the roots have fully developed vascular connections to the stem and a fully developed root cap (Ritchie, 1994).

### **Propagation from cuttings**

The propagation of deciduous hardwoods most commonly utilizes stem cuttings that are manipulated chemically, mechanically and environmentally to induce root and shoot formation. In most cases this results in a clone of the parent material. Cutting propagation has been used for many years in the ornamental horticulture industry as the preferred method of propagation to ensure that the next generation is genetically identical to the parent material, ensuring the preservation of desirable traits (Hartman and Kester, 2011). Factors such as location of cutting on plant, time of year taken and stage of growth can have great effect on the success rates (Hartman and Kester 2011).

Woody plant stem cuttings can be divided into three groups: hardwood, semi-hardwood, and softwood (Hartman and Kester, 2011). Hardwood cuttings consist of mature dormant firm wood collected after the leaves have abscised (Fourrier, 1984). Hardwood cuttings are collected from last year's growth during the dormant season any time from late fall to early spring with collection times usually being species specific (Fourrier, 1984). Given that hardwood cuttings are dormant when cut, they possess a number of advantages that have made this type of stem cutting very popular in the horticultural industry. The ease of rooting and robust nature of some hardwood cuttings make them more cost effective compared to other cuttings. Hardwood cuttings are generally easy to root, are not readily perishable, and are easily transplanted and easily shipped (Fourrier, 1984; Hartman and Kester 2011).

Hardwood cuttings are often taken in the fall when leaves can be pulled from the stem without tearing the bark, with cuttings generally selected from young, healthy, exterior shoots grown in full sun. The size of cuttings is species dependent and ranges from 10 to 76 cm with a diameter ranging from 0.6 to 2.5 cm. Shoots smaller than 0.6 cm diameter are thought to not contain enough stored carbohydrates to support the production of adventitious roots and shoots and are therefore avoided. Tip cuttings are usually avoided due to limited carbohydrate storage and the fact that terminal buds usually contain unwanted flower buds. The typical hardwood cutting usually consists of two nodes with the bottom cut just below the bottom node and the top cut 1.3 to 2.5 cm above the top node (Hambrick et al., 1991; Hartman and Kester, 2011).

Semi-hardwood cuttings are made from leafy summer and early fall cuttings of deciduous plants with partially mature wood (Hartman and Kester, 2011). Cuttings are collected in the early morning hours when cuttings are turgid and to reduce evapotranspiration related stress. Care is taken to ensure that cuttings are kept cool and moist to prevent water loss. It is considered good practice to plant cuttings on the same day they are collected. When this is not possible, cuttings can be stored for a limited time under refrigeration. Cuttings are generally 7.5 to 15 cm long with leaves retained at the upper end of the cutting; if the leaves are too large, one third to one half can be removed to reduce leaf surface area. In contrast to hardwood cuttings, shoot terminals of semi-hardwood cuttings root more readily, however, for some species, lateral cuttings are used as well. (Hartman and Kester, 2011).

Semi-hardwood cuttings are more fragile than hardwood cuttings, thus requiring additional assistance to ensure proper rooting. Rooting usually occurs in a temperature controlled environment with high levels of shade, and under fog or mist to ensure that cuttings do not dry out. Additional measures such as stem wounding at the base, synthetic auxin application and bottom heat are needed to stimulate rooting of difficult-to-root species (Hartman and Kester, 2011).

Softwood cuttings of woody plants consist of material collected when the growth of new shoots ranges from 2 to 8 weeks. Cuttings are taken from soft succulent new spring growth of deciduous species produced during growth flushes; thus for most deciduous species, softwood cuttings can only be collected once a year. Many difficult-to-root ornamental plants such as Forsythia, Magnolia and Weigela are started in this manner (Hartman and Kester, 2011).

Softwood cuttings generally root faster than other types of cuttings; however, due to the nature of the cutting, special care is needed to ensure survival. Cuttings are collected early in the morning to minimize evapotranspiration related stress and are kept cool until planting. During rooting, cuttings need to be kept in conditions that will prevent damaging levels of water loss from leaves, and growing

medium temperature should be maintained between 23 to 27 °C with high shade levels. Mist or fog systems are necessary and can help cuttings survive periods of high temperatures up to 32°C. Mist/fog systems along with high levels of shade have been shown to increase effectiveness of exogenously applied IBA in the rooting of many species (Zaczek et al., 1997).

### **Auxin application**

Since the discovery of synthetic auxins for use as a rooting stimulant, many application methods have been used over the years; however, in the horticultural industry only three methods are commonly used. Those methods are: the powder dip, the quick dip and the dilute soak (Blythe et al., 2007). The quick dip and powder dip are considered to be the most effective with the quick dip being the more commonly used of the two. The dilute soak is infrequently used and only with cuttings of certain species (Dirr, 1998; Macdonald, 2006).

Commercial powder preparations, which can be purchased from many horticultural suppliers, consist of a specified concentration of rooting hormone suspended in talcum powder. The powder is applied to the basal end of the cutting by first wetting the cutting stem to ensure proper adhesion then dipping the base of the cutting to apply the powder. Pre-dipping cuttings in a 50% aqueous solution of acetone, ethanol or methyl alcohol before powder dipping has shown to enhance rooting (Howard et al., 1984). The advantages of the powder dip method include ease of application, visible evidence of auxin application, no preparation required, and ease of product storage. Disadvantages include loss of auxin while sticking cuttings (can be rubbed off when cuttings are inserted into rooting media), limited selection of concentrations, limited uniformity of application and higher cost compared with quick dips (Blythe et al., 2007; Hartman and Kester, 2011; Macdonald, 2006).

The basal quick dip method has been shown to be the most effective method of auxin application and the most cost effective, making it the most widely used application method in the horticulture industry. In the quick dip method, auxin is suspended in an aqueous solution of 50% alcohol with concentrations of synthetic auxin varying from 500 to 10,000 ppm (0.05 to 1.0 percent) (Hartman and Kester, 2011). The bottom 0.5 to 2 cm of the cutting is dipped in the solution for 1 to 5 seconds before inserting the cutting into the growing medium (Blythe et al., 2007). The quick dip method has many advantages including speed of application, uniformity of application, ability to make a variety of concentrations, long term storability and cost effectiveness. Disadvantages include high cost of equipment and auxin concentrations can change over time due to alcohol evaporation or dilution from wet cuttings (Blazich, 1988; Hartman and Kester, 2011; Macdonald, 2006; Wood, 1982).

In the dilute soak method, the basal 2.5 cm portion of the cutting is soaked in a dilute solution

for periods of time ranging from 2-24 hours (Loach, 1987). Dilute soak concentrations of auxins range from 20 to 250 ppm and are used with cuttings kept at 20 °C. The dilute soak is not a common method of auxin application; however, dilute soaks allow for greater uptake of auxin and have been shown to be of benefit in hard to root cuttings such as *Prunus spp.*, conifers, evergreens and deciduous shrubs (Hartman and Kester, 2011). Dilute soak is uncommon due to several disadvantages: time required for soaking, the need for additional space and equipment, and sensitivity to environmental conditions leading to irregular results (Blythe et al., 2007; Blazich, 1988; Macdonald, 2006).

### **Wounding**

For some difficult-to-root species such as juniper, arborvitae, rhododendron, maple, magnolia, and holly, wounding the bottom 2.5 cm of the cutting has been shown to increase root development (Blythe et al., 2007). Wounding occurs when the outer bark is stripped away or penetrated to expose the cambium tissue, allowing for increased auxin absorption. Techniques for wounding include; stripping of leaves or branches at the base of the cutting, making one or more vertical incisions down the side of the cutting base, splitting the center of the stem, or removing a slice of tissue from one or more sides of the basal end of the cutting (Wells, 1963; Howard et al., 1984). It should be noted that care should be taken not to crush or otherwise damage the cutting while collecting the cutting or during wounding, as the end and the basal portions of the cutting are necessary for water uptake and adventitious root formation (Wells, 1963).

### **Rooting media**

The use of rooting media is highly dependent on the species and on growing conditions. Other factors such as cost and ease of handling determine usage; however, rooting media has four functions: to hold the cutting in place during rooting, to provide moisture, to enable air exchange and to block light transmission at the base of the cutting (Hartman and Kester, 2011).

Rooting media generally consists of two elements, an organic component such as peat, sphagnum moss or softwood or hardwood bark shavings and a coarse mineral component used to ensure large pore spaces for aeration and drainage. The coarse mineral component can include perlite, vermiculite, expanded shale, coarse sand or grit, pumice, scoria, polystyrene and rockwool (Avery and Beyl, 1991; Carter and Slee, 1991).

### **Growing conditions**

Environmental conditions necessary for successful rooting vary by species and cutting type; however, some essential conditions are required. The most common environmental conditions include a rooting media temperature of 18 to 25°C for temperate species and 25 to 32°C for tropical species,

conditions that favor low evaporative stress and maintenance of high turgor levels, moderate light levels of  $100 \text{ W/m}^2$  (varying with species), and rooting medium that is well drained/aerated and clean (Hartman and Kester, 2011).

Many techniques exist for maintenance of the above listed conditions; however, given their popularity and widespread use, this section will focus on intermittent mist/fog systems. Intermittent mist/fog systems are widely used with softwood, hardwood and semi-hardwood cuttings and are highly adaptable to meet the conditions needed for a wide range of cuttings and species. The application of fog or mist covers the medium and the leaf with a layer of water that reduces evaporative stress by intercepting light so that water is evaporated from the surface of the leaf instead of from the internal leaf tissue.

The application of fog or mist also lowers the temperature of both the cutting and medium by evaporative cooling, thus lowering water loss from the cutting. In some cases, the cooling effect of the mist/fog which keeps the leafy upper portion cool, is accompanied with bottom heat. Bottom heat is the application of external heat to the growing medium from below, most commonly in the form of warm water pumped through tubing (Hartman and Kester, 2011). The warming of the leafless lower portions of the cutting accelerates the physiological processes of root initiation and growth, resulting in shorter rooting times (Preece, 1993).

The proper application of mist/fog requires application during daylight hours to ensure a film of water covers the leaf but does not saturate the soil; achieved with a number of control and monitoring devices. Static control devices are first governed by an adjustable controller that shuts the system off during non-growing or night hours when mist is not needed, then during growing daylight hours provides mist/fog at preset intervals and durations. This system is low cost, easy to install and dependable but requires daily manipulation by the grower to account for daily fluctuations of light and temperature (Hartman and Kester, 2011).

In contrast with static systems that require daily input to adjust for changing environmental conditions, dynamic control systems incorporate plant environmental parameters recorded by an array of sensors to determine appropriate growing conditions. Dynamic control systems can gather and process information for the management of environmental conditions from an array of sensing equipment that can range from simple mechanical devices to sophisticated sensors. Screen balances provide the lowest cost option for dynamic control; a small metal mesh screen is attached to a lever that in turn controls the mist system, the weight of accumulated mist builds on the screen until the lever falls to a position that interrupts the flow of mist, thus acting as an environment dependent control for mist



application (Hartman and Kester, 2011). Photoelectric cells provide a more sophisticated method of regulating mist/fog application based on the measurement of light irradiation. The photoelectric cell converts light into electricity that is measured and used to determine when a certain concentration of irradiation has been achieved, thus activating the mist system. This system is well suited for an enclosed mist system where irradiation is the largest indicator of evapotranspiration and factors such as air movement and humidity can be more closely controlled. Photoelectric systems utilize 70% less water than static mist systems, thus conserving water but also lessening potential leaching of nutrients from cuttings (Burger, 1994; Hartman and Kester, 2011).

The aggregation of data from a number of instruments including photoelectric cells and instruments that monitor atmospheric conditions, media, cutting temperature, vapor pressure and differences in air and leaf temperature can be managed via computerized control systems that can process the data and determine interval and duration of mist/fog application. This approach, although the most costly, allows for the most efficient application of mist/fog to ensure the best growing conditions (Hartman and Kester, 2011).

Mist systems incorporate nozzles that spray high pressure water through a pressure jet or a deflection or anvil nozzle to create mist with water droplet sizes ranging from 50 to 100  $\mu\text{m}$ . At 9 to 20 liters of water per hour, pressure jets use less water than deflection or anvil nozzles, however other factors should be considered for determining suitability of nozzles, including cost, maintenance, size of mist droplet and mist pattern (Hartman and Kester, 2011).

Like mist, fog and micromist systems reduce evaporative stress by coating the leaf in a thin film of water. Micro mist systems produce droplets ranging from 2 to 100  $\mu\text{m}$  whereas fog systems produce droplets with a diameter of 40  $\mu\text{m}$  and below (Mee, 1994). True fog droplets sized 40  $\mu\text{m}$  and below will stay suspended in air, allowing for evaporative cooling and increasing the relative humidity to 93 to 100 percent. Unlike fog, the larger micro mist droplets lose suspension and settle on the surface of the cutting and media. Although this is beneficial in lowering evaporative stress, too much settling of larger diameter droplets can lead to saturation of media and nutrient leaching from the cutting leaf (Priapi, 1993). Monitoring and control of relative humidity and vapor pressure are necessary for the accurate control of intermittent fogging systems that maintain proper humidity levels without the buildup of stagnant warm air (Hartman and Kester, 2011).

### Seed stratification

Seeds are the most common propagules of higher plants. Seeds serve a number of purposes including dispersal of species over space and time and enabling survival during periods in which growing

conditions would be unfavorable or impossible (Hilhorst and Lubzens, 2010). Even though dormancy is the mechanism that allows seeds to endure for long periods of time, potentially under harsh conditions, it poses a potential problem to any grower who wishes to propagate a species via seed.

Dormancy as defined by Baskin and Baskin (1998) is a seed that cannot germinate in a specific period of time under environmental conditions that would otherwise be favorable to germination. Dormancy should not be considered the absence of germination but rather a characteristic of the seed that determines the conditions necessary for germination (Vleeshouwers, 1995; Thompson, 2000). It is important to note that seeds may be quiescent instead of dormant; a quiescent seed needs only to imbibe water at permissive temperatures to germinate (Hartman and Kester, 2011).

There exists, two forms of dormancy; primary dormancy and secondary dormancy. Primary dormancy is a condition that exists as the seed is shed from the plant as part of the developmental program (Hilhorst and Lubzens, 2010; Hartman and Kester, 2011). Primary dormancy prevents germination even during favorable environmental conditions (Hartman and Kester, 2011). Secondary dormancy occurs after seed imbibition as a result of unfavorable growing conditions (Hilhorst and Lubzens, 2010). It is possible to fluctuate between secondary dormancy and non-dormancy as seasons and environmental conditions change (Finch-Savage et al., 2006).

Primary dormancy can be further subdivided into three mechanisms: exogenous, endogenous and combinatory (Hartman and Kester, 2011). Exogenous dormancy can be classified as factors that impose dormancy outside the embryo and can be further classified into physical or chemical dormancy (Hartman and Kester, 2011). Exogenous physical dormancy is imposed upon the seed via an impenetrable seed coat or by the formation of an impenetrable layer of cells that forms on the outer integument (Hartman and Kester, 2011). This impenetrable layer, also known as palisade cells, can be made of lignin, suberin, cutin, and waxes (Egley, 1989; Rolston, 1978). Seed coat and palisade cells create a barrier to water uptake and can also limit gas exchange, leading to internal oxygen deficiency that may perpetuate dormancy (Bewley, 1997). Exogenous chemical dormancy is induced by germination inhibitors found in the fruit and seed covering tissues (Evenari, 1949). Many hormones contribute to dormancy; however, gibberellins and abscisic acid play the largest and most well understood roles in the promotion and inhibition of germination (Hartman and Kester, 2011). Much has been written on role that hormones play in the maintenance and release of seed dormancy. To obtain a better understanding of these hormones, the reader may reference Hartman and Kester (2011) as a good starting point.

Endogenous dormancy is the result of factors within the seed that cause the suspension of germination (Hartman and Kester, 2011). Three types of mechanisms control endogenous dormancy: physiological, morphological and morphophysiological. Endogenous physiological dormancy is characterized as factors within the seed that inhibit germination (Finch-Savage et al., 2006). Depth of dormancy can be used to separate endogenous physiological dormancy into three categories: non-deep, intermediate, and deep (Hartman and Kester, 2011). Both non-deep and intermediate endogenous physiological dormancy are characterized as the radical's inability to escape the seed covering and the seed respectively (Baskin, 2004). In the case of deep endogenous physiological dormancy, the embryo does not germinate when separated from the seed; or if germination occurs, a dwarf is produced (Baskin and Baskin, 1998).

In morphological and morphophysiological mechanisms, underdevelopment of the embryo at the time the seed is shed from the plant is the main cause of dormancy (Baskin and Baskin, 1998). Morphological dormancy is simply the underdevelopment of the seed at the time the seed is shed from the plant; and as the name suggests, morphophysiological dormancy is the combination of morphological and physiological dormancy (Hartman and Kester, 2011).

When considering primary dormancy, it may not be the case that one of the above mentioned mechanisms are responsible for the initiation and perpetuation of dormancy. Combinatory dormancy is the combination of multiple primary mechanisms to induce and or sustain dormancy (Hartman and Kester, 2011).

Primary dormancy has evolved to control the time and conditions for seed germination. The failure of a seed to germinate after suspension of primary dormancy can lead to the onset of secondary dormancy. Secondary dormancy prevents germination of an imbibed seed when environmental conditions are unfavorable, and can be defined as either thermo dormancy or conditional dormancy (Bewley and Black, 1994; Karssen, 1981). Thermodormancy is induced after the discontinuation of primary dormancy is followed by exposure to high temperatures. In the case of conditional dormancy, the ability to germinate is governed by the time of year (Hartman and Kester, 2011).

### **Breaking dormancy**

In disrupting or ending dormancy, stratification and scarification techniques are used to break the chemical and physical mechanisms that keep a seed dormant. Stratification is the exposure of an imbibed seed to extended periods of chilling or warm temperatures to break dormancy (Hartman and Kester, 2011). Scarification is the process of altering the seed cover via mechanical or chemical methods (Hartman and Kester, 2011).

Before stratification, seeds are soaked in water to ensure that the seed is imbibed, they are then packed into a sterile moist medium such as sand and placed in a container that will allow for gas exchange. Most seeds require a stratification period between one and four months with the usual temperature being between 1 and 10°C. In some cases, seeds can be stratified outdoors directly in the seed bed, although special care needs to be taken to prevent against loss from predation. For some seeds, alternating periods of chilling and warming may be necessary (Hartman and Kester, 2011).

Scarification uses mechanical or chemical means to alter the seed covering, allowing for the alleviation of physical dormancy imposed by a hard or impenetrable seed coat (Hartman and Kester, 2011). Mechanical scarification is achieved with the physical abrasion of the seed coat, commonly in the form of seeds being tumbled with coarse sand or gravel but other methods are possible such as the use of sandpaper or nicking the seed coat with a knife. A variety of acids or hot water are used in chemical scarification in order to abrade the seed coat, with the type of acid and length of treatment being dependent on the recalcitrance of the seed coat (Hartman and Kester, 2011).

Seed viability can be tested in three ways; standard germination, excised embryo, and the tetrazolium test. For the standard germination test, seeds can be placed in a germination medium or placed on plastic germination trays or petri dishes lined with a moist paper or blotter and germinated. The excised embryo test is normally applied to tree and shrub seeds that need extended stratification periods to germinate. To determine germination rates, embryos are removed from the seed and germinated under normal conditions. The compound, 2,3,5 triphenyl-tetrazolium (TTC) is used as a biological indicator of seed viability in the tetrazolium test; viability is indicated when the TTC turns red in the presence of dehydrogenase enzymes that result from respiration (Hartman and Kester, 2011).

When fully mature, the seed of *Pterocarya stenoptera* is fully developed excluding morphological, dormancy (Nikolaeva 1977). No evidence can be found indicating exogenous dormancy, moreover, the seed absorbs water readily when soaked for a short period of time further indicating a lack of exogenous limitations of dormancy. Studies conducted by (Grbic et al., 2011) classify dormancy of the wingnut seed as moderate or shallow physiological dormancy.

Studies conducted by (Grbic et al., 2011) indicate that one month of cold stratification in moist perlite produced a germinative capacity of 56.5%, this being the highest rate of germination among trials of one and two months stratification lengths, including naked seed stratification using the same time periods. In a study conducted by Burchell (2002) germination rates of above 95% were reported for a stratification procedure involving soaking for 3 days with water changed daily along with three months of cold stratification at 2.2-3.3 C° packed in damp peat moss. Grbic et al. (2011) were not successful in

germinating seeds that were stored for an extended period of time (one year) and seeds that were warm stratified. A similar study conducted by Cicek and Tilki (2008) with seeds of *Pterocarya fraxinifolia* indicated that 3, 5, and 7 weeks of cold stratification at 4 °C produced germination rates of 95.2, 94.3, and 93.3%, with warm stratification of 3 weeks producing only 38.5% germination. Shao (1989) indicated that *Pterocarya stenoptera* had more than a 70% germination rate when treated with temperatures varying from 15-20 °C to 30 °C before stratification (stratification length and temperature unknown).

### Biomass composition

Plant tissue is comprised of over 35 different types of cells, all with unique structure and composition (Chundawat et al., 2011). However, as diverse in structure and composition as the cells may be, all plant cells have recalcitrant cell walls that encapsulate the inner workings of the cell providing shape, rigidity and a thick (0.1 to 10 µm) barrier that provides protection from foreign bodies and pathogens (Chundawat et al., 2011). Plant cell walls are generally comprised of three distinct layers, the middle lamella, primary cell wall and secondary cell wall with the secondary cell wall having three layers (S1, S2 and S3) (Chundawat et al., 2011). In relation to plant biomass, it is primary and secondary walls that account for the vast majority of hemicellulose and cellulose with the secondary wall containing greater quantities of cellulose (Chundawat et al., 2011), making their composition, and deconstruction via mechanical, chemical and thermochemical processes of great interest to researchers.

Wood can be defined as a three dimensional biopolymer composed of interconnected networks of cellulose, hemicellulose, lignin and trace amounts of inorganics and extractives (Rowell, 2005). The cell wall structure of dried wood consists of 65 to 75% carbohydrates with another 18-35% composed of lignin (Rowell, 2005). Dry wood generally has a composition of 50% carbon, 44% oxygen, 6% hydrogen and trace amounts of inorganics (Rowell, 2005).

A complete chemical analysis of wood accounts for all organic components (Rowell, 1984) with the two major chemical components being carbohydrates and lignin with extraneous components such as extractives and ash comprising their remainder of the solid matter (Rowell, 1984). Carbohydrates found in both celluloses and hemicelluloses and the released simple sugar constituents are precursors of biofuels, with much research focusing on the energy required and cost-effective methods of rendering simple fermentable sugars from the complex structures of primary and secondary cell walls.

To date, no work has been published regarding the properties of Chinese wingnut as they related to biomass for biofuel production. Work has been carried out in Asia exploring extractive properties; of the species, enclosed here is a limited summary of these works and their nature. Extracts

from the leaves, roots and bark of Chinese wingnut have been used as a pesticide, and for medicinal purposes that include use as a carminative, anthelmintic as well as an infusion used to alleviate various skin ailments (Kuo et al., 2006). The Extract Pterocarnin A, derived from the bark, has been reported to exhibit anti-herpes simplex virus type-2 properties (Cheng et al., 2004). In addition to Pterocarnin A being useful in the treatment of herpes simplex virus type-2, work by Kuo et al., 2006 has shown that the extract Pterocarnin A was useful in disrupting the growth of specific breast cancer tumors. Outside of the potential health benefits of extracts from Chinese wingnut, work by Wang et al., (2006) indicated that water and n-butanol leaf extracts have molluscicidal effects with LD<sub>50</sub> values as low as 13.2 mg/L.

### **Cellulose**

Cellulose is the most abundant organic chemical on the face of the earth, and as such, has been the main focus in the development of next generation biofuels due to the feedstock's theoretical ability to be hydrolyzed to pure glucose (Rowell, 2005; Chang, 2007). Cellulose accounts for 15 -30% of the dry mass of the primary cell wall, up to 40% of the secondary cell wall (Sticklen, 2008) and between 40-50% of total hardwood biomass (Rowell, 2005). Cellulose is a homo-polymer of  $\beta$ -1, 4 linked glucose units, most commonly taking the form of crystalline microfibrils, but it can also be found in an amorphous form (Carpita and McCann, 2000). Two glucose anhydride units having lost two water molecules, form cellobiose, the basic repeating unit of the cellulose polymer (Mohan et al., 2006). The cellulose chain develops strong intermolecular hydrogen bonds between cellulose chains, which adds rigidity to the chain and promotes formation of the crystalline structures (Klemm et al., 2005). Bundles of cellulose molecules form microfibrils which when combined together form fibrils which finally bundle to form cellulose fibers (Chang, 2007).

Current methods of processing cellulose to make ethanol, begin with milling to reduce particle size, followed by thermochemical treatment to partially separate lignin and hemicellulose, the end result being cellulose that can be more easily digested. After pretreatment, enzymatic saccharification converts cellulose into soluble glucose monomers or oligomers that are later consumed by yeast in the fermentation process (Chang, 2007). Cellulose requires at least 4 enzymes for depolymerization into smaller units to allow for fermentation (Chang, 2007). Multiple isozymes of three different classes of cellulases are needed to conduct hydrolysis efficiently, the most common enzymes being endoglucanases, exoglucanases, and  $\beta$ -glucosidase (Chang, 2007).

### **Hemicellulose**

Hemicelluloses are comprised of low weight branched polymers arranged in a  $\beta$ -1,4-linked backbone composed of a relatively small number of sugar residues (Sannigrahi, 2010). Hemicellulose,

the second most abundant sugar-based polymer behind cellulose (Chang, 2007), and is a heterogeneous blend of polysaccharides with constituent parts being mainly pentose (xylose, arabinose) and hexose (mannose, glucose, galactose) sugars, constituting on average, 25-35% of the dry mass of hardwood (Girio et al., 2010; Saha, 2003; Rowell, 2005). Hemicellulose composition varies depending on cell tissue, plant species and other factors such as glycosidic linkages (Chundawat, 2011). The heteropolymer structure of hemicellulose is characterized by a long linear backbone of repeating sugars with short branched side chains composed of acetate and sugars (Duff and Murray, 1996). In monocots and dicots, the most abundant forms of hemicellulose are glucuronoarabinoxylans and galactoglucomannans, respectively (Chundawat et al., 2011).

The utilization of hemicellulose is essential for the efficient and cost effective production of biofuels (Saha, 2003). The current conversion of hemicellulose substrates is problematic, however methods are being developed that will allow for the full utilization of pentose and hexoses sugars and the glucose sugars of cellulose. Currently pretreatments, such as alkaline peroxide and ammonia fiber explosion, produce solubilized partially degraded hemicellulose biomass needing further degradation via enzymes to produce fermentable sugars (Saha, 2003). The development of a pretreatment method that minimizes the production of fermentation inhibitory compounds and use of a mixture of hemicellulases that are able to break down the complex sugars of hemicellulose will increase the feasibility of making biofuels from hemicellulose, in addition to the well-established production of biofuels from cellulose (Saha, 2003).

### **Lignin**

Lignin is a cross-linked three dimensional amorphous phenolic biopolymer (Sannigrahi et al., 2010) that accounts for 20-30% of woody biomass. Lignin is the second most abundant biopolymer (compared to cellulose as the most abundant) in the biosphere (Ralph et al., 2004). The lignin biopolymer is synthesized from three separate types of phenolic monomers resulting in a wide variety of bonding motifs such as, phenylcoumarans, biphenyls, and biphenyl ethers (Chang, 2007). Biosynthesis can be a very plastic process that adjusts to changes in monomer availability or it can involve utilizing phenols other than those commonly used in lignin biosynthesis (Ralph et al., 2004). However, it should be noted that the bonding process has not been fully characterized (Chang, 2007). In the cell wall, lignin binds cell fibers and vessels and also plays an important role in protecting the plant from pathogens and insects (Sticklen, 2008). Lignin is comprised of three basic monomeric units; p-hydroxyphenyl, guaiacyl, and syringyl, with prevalence and ratios of the constituents varying with species. Hardwoods are normally comprised of guaiacyl and syringyl units but can also contain small amounts of p-

hydroxyphenyls (Zhao et al., 2012). Guaiacyls are the main monomeric unit of softwood lignin, with herbaceous plants having all three basic monomeric units in varying amounts (Chundawat et al., 2011; Buranov and Mazza, 2008).

The potential chemical energy of lignin is high; however, the recalcitrance of constituent structural qualities make it the most difficult of the major cell wall components to manage in an industrial capacity (Chang, 2007). Lignin is a major barrier to enzymatic scarification of wood cellulose and hemicellulose (Zhu and Pan, 2010). Cellulases and hemicellulases are prevented from accessing the carbohydrates in cellulose and hemicellulose by the physical barrier created by the recalcitrant nature of lignin (Zhu and Pan, 2010). Therefore, methods of removing lignin from the process stream are necessary to ensure a high degree of polysaccharide hydrolysis. In addition to lignin being a physical barrier, lignin has additional detrimental effects including: non-specific absorption of hydrolytic enzymes that lead to the creation of “sticky lignin”, non-productive binding and interference of cellulolytic enzymes that lead to unwanted lignin-carbohydrates complexes, and unfavorable or toxic conditions for microorganism (Agbor et al., 2011).

Pretreatment is believed to melt lignin, allowing for coalescence upon cooling to alter its properties. The lignin can then be precipitated (Agbor et al., 2011; Brownell and Saddler, 1987; Lynd et al., 2002). Delignification causes biomass swelling, disruption of structure, increases in internal surface area and increased accessibility of cellulose fibers to cellulase enzymes. Although pretreatment may not fully remove lignin, the chemical structure can be altered sufficiently to increase its digestibility, allowing for acceptable levels of polysaccharide hydrolysis (Agbor et al., 2011).

### **Extractives**

Extractives are extraneous materials that can be extracted from wood via a solvent and they comprise 4-10% of the dry weight of species found in temperate climates (Rowell, 2005). In some cases, the extractives are classified by the solvent used in extraction (Rowell, 2005). Solvents used in extraction range from water to ether-soluble extractants, with hundreds of wood chemicals identified in this manner (Rowell, 2005). In general, extractives are found most prevalently in the cell wall and include fats, fatty acids, fatty alcohols, phenols, terpenes, steroids, resin acids, rosins, waxes and many other chemicals. Softwoods generally have higher concentrations of extractives than hardwoods (Rowell, 2005).

Extractives are typically removed from the biomass before any analytical analysis due to potential for interference with analytical methods, but can be a source of value added co-products to a large scale lignocellulosic biorefinery (Sannigrahi et al., 2010). Extractives isolated in this study are the



product of an ethanol and water extraction; however on a large scale, steam or supercritical CO<sub>2</sub> would be used to avoid the large scale usage of industrial solvents (Sannigrahi et al., 2010).

### **Ash**

Ash is defined as the residue remaining after treatment at 575 °C. Additionally, ash can be defined as inorganic elements that interfere with the conversion of biomass into biofuel, and as a result, are incorporated into a waste stream. Ash can alternatively be defined as the source of slagging or bio char in thermochemical conversion (Sannigrahi et al., 2010). A thorough understanding of ash content of a biomass feedstock and what effects ash will have on conversion of that feedstock to biofuel is necessary, regardless of the conversion pathway (Sannigrahi et al., 2010). Inorganic ash components such as P, K, Ca, and Mg are essential soil nutrients and can be used as soil amendments on the very land from which the biomass was initially removed (Paine et al., 1996).

### **Pretreatment**

In order for biomass to be converted into biofuel, the lignocellulosic biomass must first undergo pretreatment. The fermentation of hexoses and pentose sugars derived from the hydrolysis of cellulose and hemicellulose is complicated by many physiological, structural and compositional factors. The goal of pretreatment is to break down highly recalcitrant lignin structures and disrupt the crystalline structure of cellulose to allow for easy access of acids or enzymes for hydrolysis (Mosier et al., 2005). Pretreatment incorporates various techniques including biological treatment, steam explosion, ammonia fiber extraction and chemical treatment to make the constituents of cellulose and hemicellulose more accessible (Kumar et al., 2009). An ideal pretreatment process is one that produces a disrupted, hydrated substrate without producing unwanted formation of inhibitors and sugar degradation products (Agbor et al., 2011). Given the complexity of separating lignin from desirable polysaccharides, pretreatment can be the most expensive process in the biofuel conversion process; however, many approaches are being developed that will allow for increased efficiency at lower costs. The work of Kumar et al. (2009) and Agbor et al. (2011) provide a comprehensive picture of established practices and processes currently under development.

Woody biomass requires more pretreatment and is less susceptible to microbial breakdown than herbaceous material, due to its tough recalcitrant nature and higher levels of lignin (Zhu et al., 2010). Currently, it is possible to release constituent sugars from woody biomass for fermentation into ethanol; however, much work still needs to be done on converting woody biomass into fuel in a cost effective manner (Zhu et al., 2010). Current procedures and guidelines, such as those set forth by the U.S Department of Energy biofuels research road map ( U.S. D.O.E., 2005), call for mechanical biomass

size reduction to the level of fibers or fiber bundles before thermochemical pretreatment, an extremely energy intensive process. Research conducted by Lee et al. (2009) and Sun et al. (2009) indicates that energy required to physically process woody biomass down to the level of fiber or fiber bundles is roughly equal to the energy of the resulting biofuel after conversion to mechanical electrical energy, illustrating the need for a better method of biomass pretreatment (Zhu et al., 2010).

In order to reduce energy use during physical pretreatment of woody biomass, Zhu et al. (2010) recommend sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) as a pretreatment. Developed in conjunction with the US Forest Service Forest Products Lab and the University of Wisconsin-Madison (Wang et al., 2009; Zhu et al., 2009), SPORL is similar to dilute acid pretreatment with the addition of a sulfate or bisulfate catalyst (Zhu et al., 2010). The SPORL process is conducted at 160 to 190°C for 10-30 minutes in batch form with the sulfite increasing the pH (Zhu et al., 2010). Increasing the pH while using acid concentrations similar to those used in dilute acid pretreatment, markedly reduces the formation of fermentation inhibitors such as furfural and 5-hydroxymethylfurfural as compared to non-SPORL reduced acid pretreatment (Zhu et al., 2010; Shuai et al., 2010; Wang et al., 2009; Zhu et al., 2009). The sulfination of lignin softens wood chips, thus lowering the energy necessary for milling to fiber or fiber bundles (Zhu et al., 2010). If combined with steam explosion (a process where biomass is treated with high pressure steam combined with a sudden lowering of pressure enabling explosive decomposition of the biomass) as a form of pretreatment, mechanical milling is not necessary (Kumar et al., 2009). A complete explanation of the SPORL process can be found in (Zhu et al., 2010; Wang et al., 2009; Zhu et al., 2009).

### **Methods of compositional analysis**

The ability to accurately and precisely identify and measure the constituent components of biomass feedstocks is necessary to enable the large scale commercialization of biofuel. Feedstock analysis allows for accurate measurement of all constituent components, most importantly carbohydrate content which is directly proportional to biofuel yield (Aden et al., 2002) and inhibitory factors such as lignin and ash that can be detrimental to the production process (Hames, 2009).

At present, two standards of analysis for the wet chemical characterization of biomass are widely accepted by the scientific community (Hames, 2009). The International Energy Agency provides a set of methodologies available from the American Society for Testing and Materials (ASTM) (ASTM standard E870-82(2006)). The National Renewable Energy Laboratory (NREL) provides a set of methodologies that include but go beyond that of ASTM (NREL, 2012 [http://www.nrel.gov/biomass/analytical\\_procedures.html](http://www.nrel.gov/biomass/analytical_procedures.html)). These methods of analysis are based on

the fractionation of the biomass sample and the isolation of purified fractions that then can be analyzed using conventional analytical instruments (Hames, 2009). This paper uses those standards set forth by NREL.

Analysis generally involves the following sequence of steps: sample preparation such as drying and mechanical grinding, removal of nonstructural materials (extractives), and hydrolysis of structural polymers to allow for chromatographic or spectroscopic analysis (Hames, 2009). In each step of constituent analysis, the dry weight of the isolated constituent is reported as a percentage dry weight of the starting analytical sample. In order to determine the isolated constituent's percentage of the dry weight of the whole biomass sample, summative mass closure calculations are made available by NREL (<http://www.nrel.gov/biomass/pdfs/48087.pdf>). Complete analysis of a biomass sample may require up to 30 measurements to report the values of 10-15 constituents (Hames, 2009). Some degree of error exists within each of the measures, and when evaluating results, it is important to determine if variance is significant (Hames, 2009). Confidence interval limits have been set for each constituent component and are available from the National Institute of Standards and Technology (NIST) (Milne et al., 1992).

Due to the diversity and variation in chemical composition, the behavior of extractives during carbohydrate and lignin analysis is unpredictable and therefore extractives should be removed as a first step after sample preparation (Hames, 2009). Additionally, extractives can account for as much as 30% of mass closure and can be a source of incorrect summative mass closure for other constituents (Sluiter et al., 2010). The solvents and number of extractions required to remove all non-structural material from the biomass will vary with biomass type and constituents present (Hames, 2009).

In the analysis of carbohydrates and lignin, a two stage acid hydrolysis is used to first separate the individual polymers and secondly hydrolyze the polymers to simple molecules that can then be analyzed by gravimetric, chromatographic and spectroscopic techniques (Hames, 2009). The first stage of hydrolysis, exposure to 72% sulfuric acid, cleaves the non-covalent bond between biomass polymers (Hames, 2009). After initial disruption, a second dilute stage follows in which complete polymer hydrolysis occurs with limited degradation of monomeric sugars (TAPPI, 2009).

Two stage hydrolysis breaks down the biomass carbohydrates into monomer sugars with some of the monomers being further degraded to furans and lactones (Hames, 2009). The main degradation products of carbohydrates are the five-carbon sugars arabinose and xylose, and six-carbon sugars glucose, galactose and mannose (Hames, 2009). During the two stage hydrolysis, acetyl groups are released from hemicellulose along with ester bound phenolics and uronic acids. All of the constituents

released during the hydrolysis step are quantified using a variety of chromatographic techniques (Sluiter et al., 2011).

Lignin fractionates into acid-insoluble and acid-soluble material during hydrolysis, the two components are then measured separately (Sluiter et al., 2011). Acid-insoluble material is measured gravimetrically and is corrected for ash and nitrogenous material by ashing filtered acid-insoluble materials at 575°C (Sluiter et al., 2011; Hames 2009). Acid-soluble lignin found in the hydrolysis liquor is measured by UV/V spectroscopy, with the optimal wavelengths varying with biomass types (Hames, 2009). Estimations of biomass protein content are conducted using the Kjeldahl method or combustion method and use of a conversion formula found in NREL wet chemistry methods referenced previously.

Biomass ash content is measured twice with one measurement being taken as a whole of the biomass and again on that remaining after extraction (Sluiter et al., 2005). Ash is determined by ashing the sample at 575°C and recording the weight of inorganic material remaining (Sluiter et al., 2005).

NREL has established a set of guidelines for the use of high performance liquid chromatography (HPLC) for the detection of carbohydrates in biomass hydrolysates. The use of HPLC has advantages such as simple sample preparation and simple isocratic separation with water as the mobile phase and a refractive index detector (RID) (Sluiter et al., 2011). The use of a lead cation ( $Pb^{2+}$ ) exchange column allows for precise detection of common biomass sugars (glucose, xylose, galactose, arabinose and mannose) (Sluiter et al., 2011).

In order for biomass compositional analysis to be carried out on the scale and in the time frame needed to monitor biomass feedstocks for any large biofuel operation, rapid biomass analytical methods will need to be fully developed (Hames, 2009). Traditional wet chemical analysis methods currently in use are too time consuming and costly (\$2,000-3,000 per sample) to be of any use in process control (Hames, 2009). New methods of rapid biomass analysis are being developed with the ability to analyze samples quickly and in a much more cost effective manner, approximately \$20 a sample (Hames, 2009). Near infrared (NIR) spectroscopy offers many advantages over other spectroscopic analysis techniques. The use of NIR spectroscopy allows for analysis of bulk biomass, minimizing many aspects of sample preparation. Equipment for NIR spectroscopy is commonly available and many industrial techniques developed for use by the chemical, food processing and agricultural industries can easily be adapted for use in biofuel industry (Sluiter et al., 2010). The reader is encouraged to review Hames et al. (2003) and Scaff et al. (2006) to improve understanding of NIR spectroscopy and its use in biomass compositional analysis.

The conversion of lignocellulosic feedstocks into fermentable sugars presents the largest barrier to the cost effective production of biofuels. Currently much research designed to overcome the recalcitrance of the cell wall is being conducted; however, it seems that much of this research does not account for limitations facing the large scale production of biofuel. Research needs to take into account not only the physical/chemical barriers to the production of lignocellulosic biofuel, but needs to consider other such barriers such as: feedstock availability and reliability, environmental impacts, cost and technical feasibility or particular pretreatment methods and supply chain logistics. The aforementioned list is not comprehensive but rather should illustrate the need to conduct research that would be applicable to safe and reliable production on a large scale.

# P ropagation

## Clonal propagation

### Materials and methods

Ease of propagation is essential for the development of any bioenergy feedstock crop. Asexual propagation is important when improvements in germplasm need to be saved and passed on to future crops. Sexual propagation of a potential bioenergy feedstock crop is important for breeding efforts and to preserve genetic diversity in the crop. This section includes experiments on the asexual propagation of *Pterocarya stenoptera*, Chinese Wingnut, followed by sexual or seed propagation experiments. The objective of the asexual or clonal propagation study is to determine if propagation is possible with hardwood and semi-hardwood material collected from terminal and subterminal stem segments treated with varying levels of IBA in both liquid and quick dip form. The objective of the sexual or seed propagation experiment is to determine the proper length of stratification to ensure satisfactory germination rates.

### Hardwood Cuttings

Hardwood cuttings were collected from young field-grown plants located on the University of Illinois energy farm located in Urbana, IL. In all cases, cuttings were collected on the same day as placement in the mist house. The cuttings were collected and placed in plastic bags inside insulated coolers to prevent desiccation. Cuttings were taken when plants were judged to be large and mature enough to produce cuttings of sufficient diameter and length for rooting studies. Cuttings ranged in size from 10 to 15 cm long, usually incorporating two internodal spaces and were 0.5 to 0.75 cm in diameter.

Hardwood cuttings were collected from cutting block populations on 9 Mar. 2011 and 14 Mar. 2011 for first trials of terminal and subterminal cuttings, respectively; and on 29 Feb. 2012 for both first and second trials of hardwood terminal and subterminal cuttings. This population was grown from seed sourced from the USDA North Central Region Plant Introduction Station located in Ames Iowa, seed lot number PI596388. The seed was originally collected from the wild at Kwangnung Forest Research Station, Kyong Gi Do, Namyeongju Gun, South Korea growing along a stream in a flood plain. The seed was stratified in moist sand at 4.4 °C starting in June of 2009 with seed then being planted at the University of Illinois Plant Care Facility greenhouse in September 2009, and grown for approximately one

season. The seedlings were transplanted to the field in May of 2010 and watered as needed the first growing season. Weed control included Treflan herbicide (Dow AgroSciences, Indianapolis, IN) (2.3L/ha) followed by Devrinol (United Phosphorus, Inc. King of Prussia, PA.) (9 kg/ha) the first field season and Scotts Ornamental Herbicide II (The Scotts Company, Marysville, OH) (1.12 kg/100m<sup>2</sup>) in seasons two and three.

The soil composition at the energy farm where transplants were planted is characterized by the National Cooperative Soil Survey (<http://websoilsurvey.nrcs.usda.gov/app/HomePage.htm>) as a moderately well drained Catlin silt loam with a 2-5% slope, a water table depth of 0.6-1.1 m and a depth of 2 m to restrictive feature. The location has an annual rainfall of 83-102 cm and 150 to 180 frost free days.

The cuttings in the first and second hardwood terminal and subterminal trials were subjected to wounded (a single 2- to 3-cm long sliver of bark was removed from the basal end of the cutting exposing the cambium) and non-wounded treatments. Following the wounding treatment, the basal 2 to 3 cm of the cuttings were immersed for five seconds in 0, 1000, 2500, and 5000 ppm treatments of IBA (Indole-3-butyric acid (C<sub>12</sub>H<sub>13</sub>NO<sub>2</sub>)) with 25% ethanol in distilled water. Treated cuttings were given approximately 30-60 seconds of time to allow for the solution to penetrate into the stem before placing cuttings into the rooting media. Cuttings were placed into 31.1 x 31.1 x 8.9 cm Monarch perforated flats (Salida, CO) with individual treatments being randomly placed into one of 4 quadrants, allowing for 4 treatments per flat. Growing medium consisting of a 1:1 mix (v:v) of peat and perlite (steam pasteurized).

The first hardwood cutting trials of both terminal and subterminal material consisted of nine cuttings for each of eight replications, for a total of 72 individuals per treatment. The second hardwood terminal and subterminal trials consisted of four cuttings for each of eight replications, totaling 32 individuals per treatment. For both the first and second trials of hardwood cuttings, cuttings were placed in 16 subdivided rooting flats with eight flats of terminal cuttings and eight flats of sub-terminal cuttings (separate experiments). Treatments in all trials were assigned to flats in a randomized complete block experimental design, with a single block being composed of two flats each containing four treatments, and each mist bench containing either terminal or subterminal cuttings. The two flats comprising each block were linked together in the rooting bench as dictated by the experimental design. Rooting percentage and number of roots produced by each cutting determined the effectiveness of each treatment. All studies were duplicated (2 trials) in order to validate results.

### **Semi-hardwood cuttings**

The cutting block used to supply semi-hardwood cuttings was located at the University of Illinois Plant Sciences Laboratory greenhouse facility, Urbana, IL. Semi-hardwood cuttings were collected from two generations of greenhouse grown material. Seed for the first trials of terminal and subterminal cuttings were planted on November 19, 2010 and seed for the second trails were planted In February of 2010. Seed used for both plantings originated from USDA North Central Region Plant Introduction Station located in Ames Iowa, seed lot number PI596388.

Stock plants were watered as needed and grown under high pressure sodium lights (1000W GE Lucalox bulb, Cleveland, OH), positioned approximately 1.8m above the tree canopy, providing approximately 250 umol PAR supplemental lighting. Trees were fertilized with Scotts Brand Peters Professional water soluble fertilizer having a composition of 20N-8.76P-16.6K with M-77 micronutrient system (The Scotts Company, Marysville, OH). Fertilizer was applied weekly at a rate of 250 ppm N. Conserve SC (Dow AgroSciences, Indianapolis, IN) (2 ml/gallon) was applied once for a minor infestation of thrips. The greenhouse was maintained with a heating target of 23°C and a cooling target of 28°C.

The semi-hardwood cuttings were collected in same manner as hardwood cuttings, including length and caliper of cutting. Leaves were completely removed from lower buds and approximately  $\frac{3}{4}$  of the remaining leaf area was removed to lessen transpiration demand. Cuttings consisted of a set of fully expanded leaves with one or two leaves near the terminal bud still in various stages of expansion. All semi-hardwood cuttings in the first and second trials were subjected to wounding as described above. The IBA treatments included 0, 1000, 2500, and 5000 ppm IBA in quick-dip solutions (25% ethanol) with additional treatments of 1000, 3000 and 8000 ppm Hormodin rooting powder (ohp industries, Mainland, PA).

The first and second trials of semi-hardwood terminal and subterminal cutting treatments consisted of 4 individual subsamples per treatment with eight replications totaling 32 individuals per treatment. Both semi-hardwood trials utilized a randomized complete block design. The terminal and subterminal trials were handled as separate experiments.

### **Mist house conditions**

Cuttings were rooted in the mist room of the University of Illinois Turner Hall greenhouse complex located at Urbana, IL. The overhead mist system was controlled by Phytotronics Inc. Model 12A intermittent mist controller (Earth City, MO) with the interval and duration of application adjusted to suit daily growing conditions and to ensure that cuttings did not dry out while at the same time



preventing the growing medium from becoming water logged. During the summer months, the greenhouse glass was coated with white wash and a 50 % shade cloth was placed over the bench at a height of 91 cm over the cuttings to lower evaporative stress. The mist house was equipped with additional lighting in the form of 60 watt incandescent bulbs that served to extend photoperiods when rooting occurred during short days.

For the first hardwood terminal and subterminal trials and the first trial of the semi-hardwood cuttings, mist was provided by Netafim Vibro-Mist overhead misters (Fresno, CA), with an output of 5.7 gallons per hour (GPH) at 30 psi, with overlapping spray patterns. For the first trial of the semi-hardwood terminal and for the second trial of hardwood and semi-hardwood terminal and subterminal cuttings, Netafim Fogger Nozzles which produce a 90-micron fog particle at 60 psi at a rate of 8.1 GPH were used.

Greenhouse temperature set points were 26.5°C day and 23°C at night. The actual daily average temperature was 26°C, with an average low of 23°C and average high of 28°C, charts showing high, low and daily mean temperature can be found in Tables A.1-A.7.

Due to perceived variability in rooting among the various blocks following the first set of trials for hardwood terminal and subterminal cuttings (see results), the mist system was calibrated. During the first trials it was noticed that the mist system did not appear to be providing complete and consistent coverage. The configuration of the mist nozzles did not appear to provide adequate mist to cuttings in the corners of the benches and in some sections between mist nozzles; the lack of mist in some sections potentially leading to higher mortality rates.

In order to quantify the adequacy of mist coverage, data was collected on output from mist emitters, and mist coverage. To measure mister output, a glass beaker was attached so that when activated, the mist was collected by the beaker. To measure mist coverage, 14 small plastic weight boats were placed on the bench in the same pattern as that of the propagation flats on benches 4, 5, and 10 (Table A.8), after running the mist system continuously for 3 minutes, the weight of each boat was recorded. Measurements were repeated three times and standard deviations calculated.

### **Data collection and analysis**

After studies were run for a period of at least 42 days, the flats were removed from the mist benches, the roots washed and plants rooting percentage and number of adventitious roots recorded. All analyses were conducted in R (R Core Team, 2012). Rooting percentage for each experiment was analyzed through Analysis of Deviance. Where overdispersion was detected, a quasibinomial distribution was specified for the model (Crawley, 2013; Crawley, 2005; Fox and Wiesberg, 2002).

Hardwood cuttings produced rooting percentages well below that of semi-hardwood cuttings, too low to allow for analysis of root count; therefore, only analysis of rooting percentage for the hardwood cuttings will be presented in this paper. Root count data was analyzed through Analysis of Deviance using a quasi-poisson distribution to account for overdispersion. Both rooting percentage and root count are count data consisting of integers warranting analysis of deviance unlike continuous measures such as height and mass that can be analyzed with analysis of variance. Mean separation for significant treatment factors was conducted as a series of Tukey contrasts. When considering the success of the cuttings, and with the ultimate goal of recommending of the most effective treatment, more weight was given to the number of roots produced by each cutting over rooting percentage. The mean number of roots per cutting gives an idea of how successful the cutting will be once removed from mist. If the grower were only to consider the rooting percentage, the grower would be counting cuttings that had produced one small root as rooted. Cuttings with a higher mean number of roots would be more likely to survive removal from mist and the subsequent transplant to a larger container making number of roots per cutting a better indicator of a treatment's effectiveness.

## Results

### Hardwood terminal cuttings

For both the first and second hardwood terminal cutting trials, wounding and IBA treatments were not significant. Rooting percentages in the first trial of hardwood terminal cuttings were much lower than those of the second trial (Figs. 1 and 2). First trial rooting percentages were below 50%, except for cuttings that received wounding with 2500 ppm of IBA (64%), as compared to second trial rooting percentages that were all above 50%. The increased rooting percentages in the second trial may be explained by improvements to the mist system between the two trials (see below) or by some other physiological difference in the cuttings. Blocking was highly significant in the second trial of terminal hardwood cuttings, where blocks 1 and 2 performed similarly with same being true for the following pairs of blocks: 3 and 4, 5 and 6, and 7 and 8 (Fig. 3). Table A.8 shows the block layouts on the mist benches. Blocks one through four were located on the same mist bench with block one and two located on the west end of the bench and blocks three and four located on the east end of the bench. The same pattern of block distribution applied to blocks five through eight located on bench two with five and six on the west side and blocks seven and eight on the east side. When looking at these larger groups composed of two blocks, a clear trend appears. Blocks 3 and 4 at the east end of bench 2 outperformed all other blocks while 1 and 2 and, 7 and 8 performed similarly and 5 and 6 performed poorly with

rooting rates near 40%. This clearly shows that factors other than treatment strongly affected rooting percentages in each experiment.

### **Hardwood subterminal cuttings**

Analysis of deviance indicated that rooting percentages in the first trial of hardwood subterminal cuttings were significantly affected by IBA treatment ( $\alpha=0.001$ ) (Fig. 4), and not significantly affected by wounding with no interaction between wounding and IBA treatment. Given that wounding was not significant, wounding and non-wounding results were combined in Figure 4 illustrate the significant effect of treatment. Cuttings treated with 2500 and 5000 ppm IBA showed the highest rooting percentages (35% and 24% respectively) in trial one (Fig. 4). In the second trial of hardwood subterminal cuttings, wounding was highly significant ( $\alpha=0.001$ ) with IBA treatment not being significant. When examining the rooting percentages for second trial hardwood subterminal cuttings (Fig. 5), it is clear that the wounded treatments outperformed the non-wounded treatments and that rooting percentages of the wounded cuttings appeared to increase with increasing IBA levels.

Due to the patterns of blocking effects on rooting in many of the first trial experiments, an analysis of the mist system revealed some uniformity problems. Mist emitter output rates ranged from a low of 7.7 gallons per hour to a high of 10.3 gallons per hour. Mist coverage measurements ranged from 1.6 g to 6.2 g per 25 sq. cm. Standard deviation was also calculated for the 14 weigh boats on each of the three growing benches with values of 1.4, 1.36 and 0.84 on benches 4, 5, and 10.

The tests of mist output and coverage clearly showed that mist coverage was non-uniform, starting with the rate of flow of the lowest emitter being only 74% as that of the highest output emitter. The heterogeneous output of the mist emitters was only compounded by the uneven application of mist, with an almost four-fold difference between the areas of high and low mist application rates. The discrepancies of mist application led to conditions where some flats were wetter than optimal and others received inadequate mist, resulting in higher rates of mortality.

Variability in mist coverage during the first trials of hardwood terminal and subterminal cuttings prompted the continued use of blocking in all subsequent experiments. Following the analysis of the mist system, several changes were made, including a change of emitter type and emitter number to even out the mist coverage. Rooting percentages were noticeably higher in the second trials of both hardwood terminal (Fig. 2) and subterminal (Fig. 4) cuttings. This is most likely due to the changes made in the misting system after the completion of the first trials.

### **Summary of hardwood cuttings**

When examining all four sets of hardwood cutting data, we see that second trial rooting rates were generally higher than the first trial rates. Second trial terminal cuttings produced a generally higher rooting rate (50-60%) than the first trial terminal cuttings which produced rooting percentages ranging from approximately 20-60%. Similarly, the first trial subterminal cuttings produced rooting rates ranging from 3-35% versus second trial subterminal cuttings that produced rooting percentages ranging from 3-78%. For both trials of subterminal cuttings, wounding increased rooting percentage, however in terminal cuttings, wounding yielded non-significant results. The effect of IBA on rooting percentage was only significant in the first trial of subterminal cuttings. Overall rooting percentage of both terminal and subterminal hardwood cuttings was low and inconsistent, most likely strongly affected by difficulty in rooting this type of cutting for this species and factors other than prescribed treatments. Root production (numbers of roots) data for hardwood cuttings will not be presented in this paper as overall rooting percentage and root production was much lower than that of both cutting types of semi-hardwood material. The effect of wounding on rooting percentage was only studied for hardwood cuttings and was only significant in the second trial of sub-terminal cuttings, where wounding increased rooting percentage. The beneficial effects of wounding on the hardwood cuttings, resulted in use of wounding in all semi-hardwood cutting experiments.

### **Semi-hardwood terminal cuttings**

Rooting percentages of semi-hardwood terminal cuttings were analyzed with analysis of deviance, accounting for overdispersion. For the first trial of semi-hardwood terminal cuttings, IBA treatment was significant ( $\alpha = 0.001$ ) but blocking was not. Rooting percentages ranged from 43% for the water control, to 97% for 8000-ppm talc powder treatment, with all but the 1000 ppm IBA and water control being excluded from the first significance group. (Fig. 6). IBA treatment was significant ( $\alpha = 0.001$ ) for the second trial of terminal semi-hardwood cuttings (Fig. 7), as was blocking ( $\alpha = 0.01$ ) (Fig. 8). Rooting percentages in the second trial appeared to be strongly related to rooting bench assignment. A visual comparison of Figs. 6 and 7 appears to indicate that for semi-hardwood terminal cuttings, increasing IBA concentration does not appear to consistently improve rooting percentage, leading to mixed results.

Root production (number of roots per cutting) in semi-hardwood cuttings was significantly affected by IBA treatment in both the first and second semi-hardwood terminal cutting trials (Figs. 9 and 10). For first trial semi-hardwood terminal cuttings, the most productive treatments were 8000 ppm IBA in talc along with 5000 and 2500 ppm IBA in ethanol/water, producing 8.7, 6.9, and 4.9 roots per

cutting, respectively. The 8000-ppm talc treatment produced more than 8 roots per cutting in the first trial of semi-hardwood terminal cuttings (Fig. 9), while that same treatment produced just over 3 roots in the second trial (Fig. 10). Second trial semi-hardwood terminal cuttings produced relatively few roots with little variation among treatments, with 8000 ppm IBA in talc, producing 3.4 roots per cutting, which was not significantly different from the next 3 treatments in descending order: 1000 ppm in talc, 5000 ppm in ethanol/water, and 1000 ppm in ethanol/water.

Root numbers were not significantly affected by blocking ( $\alpha = 0.056$ ) for the first trial terminal cuttings. This low p-value suggests that there may have been some gradient between two of the mist benches. Root numbers in the second trial terminal semi-hardwood cuttings were significantly affected by blocking ( $\alpha < 0.001$ ). When examining the root production in second trial terminal semi-hardwood cuttings (Fig 11), a clear pattern is visible, with blocks one through four located on bench one producing fewer roots per cutting compared to blocks five through eight located on bench three.

### **Semi-hardwood subterminal cuttings**

rooting percentages for subterminal cuttings are higher than for terminal cuttings, with 6 of the 8 treatments having 100% rooting in the first subterminal study. Given such high rates of rooting, there was not enough variation in the data set to test for analysis of deviance. For the second trial of semi-hardwood subterminal cuttings, rooting percentage was high for most treatments, but not significantly impacted by IBA treatment. Blocking however, was highly significant ( $\alpha = 0.001$ ). Rooting percentages in the second trial appeared to be strongly related to rooting bench assignment.

Indole-3-butyric acid significantly affected the number of roots produced in the first trial of subterminal semi-hardwood cuttings (Fig. 15). The effect of IBA in second trial subterminal cuttings was non-significant at the 0.05 level but with a p-value of 0.057, however for the purposes of this study, the root production for second trial subterminal cuttings will be considered significant.

Subterminal semi-hardwood cuttings in the first trial treated with 5000 ppm IBA in ethanol/water, 2500 ppm in ethanol/water, and 8000 ppm in talc produced the highest number of roots of all semi-hardwood cuttings, ranging from 20.4 to 16.8 roots per cutting (Fig. 15). Second trial subterminal semi-hardwood cuttings produced 3 roots per cutting in the 5000 ppm IBA in ethanol/water treatment which was similar to all other treatments except for the 1.4 roots per cutting produced with the ethanol/water treatment (Fig. 16).

Blocking had no significant effect on root numbers in the first trial of subterminal semi-hardwood cuttings, whereas blocking was highly significant in the second trial ( $\alpha < 0.001$ ). A gradient is visible when examining the second trial subterminal root production (Fig. 17) with blocks one through

four located on bench eight (Appendix, J) producing approximately 1 root per cutting as compared to blocks 5 through eight located on bench 8 producing between three and five roots per cutting.

### **Summary of semi-hardwood cuttings**

When considering all four iterations of the semi-hardwood trials (Figs. 6, 7, 12, 13) it is clear that the subterminal material had higher rooting percentages than the hardwood cuttings. The highest collective rooting percentages were found in the first trial semi-hardwood subterminal material with 100% rooting for all but two of the treatments. There was a strong effect of mist bench assignment on rooting percentages in the second trials of the terminal and subterminal semi-hardwood cuttings. The presence of such a large fluctuation in rooting rates suggests bench level factors such as temperature or air movement strongly affected rooting percentages. Charts showing the temperature of the mist house during the duration of the studies can be found in the appendices A-F.

### **Summary of cutting experiments**

The effects of IBA treatment on rooting percentage was studied on terminal and subterminal cuttings of both hardwood and semi-hardwood cuttings with mixed results. For hardwood cuttings, only the first trial of subterminal cuttings was significantly affected by IBA treatment, with higher levels of IBA leading to higher rooting percentages. Based on the results from this study, the treatments that are most likely to produce the best rooting percentages on hardwood cuttings would include the use of wounded subterminal cuttings along with application of IBA levels at or above 2500 ppm in 25% ethanol/water.

Rooting percentages of first and second trial semi-hardwood cuttings were significantly affected by IBA, but the positive correlation between IBA concentration and rooting percentage did not appear to be as strong.

In all semi-hardwood studies, except the second trial of sub-terminal cuttings, IBA levels significantly affected root production with a strong positive relationship between IBA level and root production. In the first trial of semi-hardwood cuttings, subterminal cuttings produced rooting percentages of 100% for most treatments. In some experiments, but not all, the treatments producing the best rooting percentages also produced the greatest number of roots per cutting. No discernible differences were detected between the use of talcum powder and water/ethanol based carriers in terms of rooting percentage and root production. Based on the results of this study, the preferred method of propagation of *Pterocarya stenoptera* by cuttings would be to utilize wounded, semi-hardwood, subterminal cuttings treated with IBA levels ranging from 2500 to 8000 ppm under mist conditions.

When considering the outcomes of each study other factors outside of mist application and environmental conditions such as temperature need to be considered. A number of factors can determine the successful rooting of both semi-hardwood and hardwood cuttings including cutting type, timing of collection, nutrient levels and carbohydrate to nitrogen ratios and cultural practices.

Successful cuttings have sufficient levels of nutrients and carbohydrates stored in tissue to allow for rooting before nutrients are depleted, however the application of mist accelerates this process (Hartman and Kester, 2011). In part, the ultimate failure of some of the cuttings to take root may have been that nutrients were leached from the cuttings before adventitious roots were formed. According to Blazich et al., (1983) prolonged exposure to mist leaches N, P, K, Ca, and Mg. One might think that an application of supplemental nutrients would correct or delay leaching to the point of mortality, however the presence of supplemental nutrients applied via the mist system or applied in any other form such as mixed in the growing medium cause sanitation issues such as stimulated algae growth (Keever and Tukey, 1979).

Given the results observed in this study it is plausible to assume that subterminal cuttings had higher stores of carbohydrates and nutrients in tissues due to their larger diameter. Both rooting and root production might also have been favored in subterminal cuttings of both hardwood and semi-hardwood by the absence of the terminal bud. Along with having lower amounts of stored nutrients and carbohydrates, terminal cuttings may contain flower buds that are unnecessary for propagation and consume valuable tissue-stored nutrients (Hartman and Kester, 2011).

In addition to nutrient leaching, the ratio of carbon to nitrogen ratio can play a large role in rooting. For most cutting stock, a ratio of high tissue stored carbohydrate to moderate to low tissue stored nitrogen is desirable. The presence of too much nitrogen such as would be present in the tissue after supplemental fertilization before cuttings are taken from stock plants would allow for excessive flushing of shoots, this would create an undesirable root to shoot ratio (Rein et al., 1991; Welander, 1995). Hardwood cuttings collected from the energy farm did not receive supplemental fertilization before cuttings were collected, semi-hardwood cuttings that were collected from the cutting block maintained at the University of Illinois greenhouses did receive fertilization on a regular basis. Proximity to cutting collection after fertilization was not tracked leading to a potentially confounding factor.

Location of collection from the stock plant can play a significant role in the success and form of the cutting. Work by Blazich et al. (1983) and Steele et al. (1990) illustrate that lateral cuttings collected from Fraser fir, white pine, Norway spruce and Sitka spruce all produced higher percentages of rooted cuttings than terminal cuttings. If further work were to be conducted on Chinese wingnut a study

testing the success rates of cuttings collected from different terminal and lateral locations on the stem might prove useful.

Results indicate that hardwood cuttings collected in February and March did not root well. Semi-hardwood cuttings that were collected at various times during the growing season in some cases rooted well and produced a satisfactory number of roots. It is well known that the season in which the cuttings are collected plays a large role in the success of the cutting. Hardwood cuttings may not have performed well because of the time of year at which they were collected. Hartman and Kester, 2011 indicated that the collection of cuttings in late winter or early spring can be problematic. Bud rest is broken by long periods of winter chilling allowing for flushing as soon as cuttings are exposed to warmer temperatures, thus not allowing for adequate time for adventitious root development. Collection in early winter or late fall may have allowed for better rooting and root production of hardwood cuttings by allowing for longer exposure to mist conditions before bud break.

Finally growing conditions in the mist house generated a number of potential confounding factors. Hardwood cuttings were rooted during the months of February and March when it was possible to collect cuttings from the cutting block maintained at the University of Illinois energy farm. The mist house was not equipped with grow lights, photo period was extended with the use of incandescent bulbs, however this did not fully compensate for the lack of natural sun light. This lack of natural light is especially striking when considering the conditions the semi-hardwood cuttings were rooted under. For first trial semi-hardwood subterminal and both second trial terminal and subterminal semi-hardwood cuttings were rooted in July. First trial semi-hardwood terminal cuttings were rooted in November due to an equipment failure resulting in 100% mortality of the first attempt in July of 2011. One might suspect that results from the first and second trial of semi-hardwood terminal would be strikingly different however they are not with both studies having similar rooting and root production results, direct statistical comparison is not possible given that both studies were carried out separately. It is not possible to know how varying conditions in the mist house affected to outcome of each study, if this study were reproduced the author would place great emphasis on removing variation from environmental conditions, time of year collected, stock plant condition, and mist application.



## Seed stratification

### Materials and Methods

In order to determine the optimal stratification period for *Pterocarya stenoptera*, a replicated study was conducted with batches of seeds being removed from stratification and planted every 5 days for a period of 60 days. Prior to stratification, a batch of 600 seeds was soaked in aerated room temperature water for three days with water changed daily. Seeds were sourced from USDA North Central Region Plant Introduction Station located in Ames Iowa, seed lot number PI596388. On 18 Feb. 2011, the seed was placed in a large plastic bag with moist, heat-pasteurized sand and placed in 4.5°C refrigeration in the dark. The first batch of 30 seeds (no stratification) was planted in Fafard Superfine Germination Mix on 18 Feb. 2011. Additional lots of 30 seeds were removed from stratification and planted every 5 days thereafter for a period of 65 days. Seeds were planted in germination trays at a depth of 1 cm. Seeds were germinated at the Plant Sciences Laboratory greenhouse facility located in Urbana, IL with set heating and cooling set points at 26.5°C for day time and 23°C for night temperatures. Germination data was collected at days 45, 55, 73 and 94 after planting. The stratification experiment was repeated with seeds from the same batch (kept dry refrigerated), approximately one year later in 2012. Given that survival data was binomial, a logistic regression model was fit to assess the impact of stratification period on seed germination. This was conducted in R using the `glm()` function (R Core Team, 2011). To account for overdispersion, a quasibinomial distribution was specified for the model (Crawley, 2013; Crawley, 2005; Fox and Wiesberg, 2002).

### Results

The results from trials one and two, presented in Table 1, show an increase in germination rates with increased days of stratification up to 30 days, with only minor changes after 30 days. The correlation between length of stratification and germination rates for the second trial was not as strong as that of the first trial. It is important to note that at no point in either trial did 100% of the 30 seeds germinate and produce a viable seedling; possibly due to the quality of the seed received. The seeds utilized in the second trial were from the same batch of seeds (stored dry refrigerated), and thus were a year older than those from trial 1. Lower germination rates in trial 2 could be explained by seed age.

Data analysis generated the probability of a single seed germinating, with the probabilities generated by the analysis combining the data from the first and second trials (Fig. 18). The results range from a probability of 0.228 for zero days of stratification to a high of 0.702 probability at 50 days of

stratification. A clear gradient of an increasing probability of germination can be seen starting at day 0 and peaking at day 50, then declining thereafter suggesting that periods longer than 50 days are detrimental.

The germination tests carried out in this study were designed to determine the optimal stratification time and did not take into account length of storage prior to testing or storage conditions. As mentioned previously, a similar germination test carried out by Burchell, 2002 produced a germination rate above 95%. Stratification procedures were similar along with length of stratification leading the author to assume that the seed used in Burchell's study was stored under more favorable conditions, possibly including a more appropriate length of dry storage that would allow for higher germination rates when germinated.

The Association of Official Seed Analysts, 1993, recommends that to test for germination rates of pure seed, four samples of 100 seeds each be tested simultaneously, if any of the lots varies by more than 10%, the test is to be discarded and retested until all samples exhibit similar germination rates. Testing seed germination in this manner provides assurances that seeds were germinated in uniform conditions. Given that an optimal stratification length has been established, a study that examines storage and drying conditions that utilizes the above method of germination testing could provide a more exact recommendation regarding seed handling and storage best practices.

In the course of conducting this study, the seeds that failed to germinate were not examined to determine why they did not germinate. A careful examination of seeds that failed to germinate could provide information as to whether the seeds were not viable, hard seeds or still dormant. Hard seeds are free of mold but have failed to absorb water, dormant seeds are also free of mold but have absorbed water but failed to germinate (Hartman and Kester, 2011). Knowing the proportion of hard and dormant seeds would indicate that some portion of the process to break seed dormancy was inadequate and if the number of dormant seeds was high enough, the dormancy breaking procedures may need to be readdressed.

# Biomass composition

## Materials and methods

Two-year-old (1998) and three-year-old (1999) Chinese wingnut seedlings were distributed to horticultural research facilities and arboreta as part of the USDA NC7 ornamental plant introduction program. Trees resulting from this distribution around the Midwest were sampled in early 2012 to determine compositional analysis as well as estimated above-ground biomass as it varied with plant location. Soil samples were also collected at each location to aid in the interpretation of growth rates.

Above ground dry biomass was calculated using models created by (Lambert et al., 2005) and (Ung et al., 2008), that use tree height and circumference at 1.3 m or breast height. All estimates include biomass of main stem(s), branches and bark. Samples of wingnut biomass, originating from a common provenance, were collected in January of 2012, at several locations in the Midwest and from a population grown at the University of Illinois to help gain a better understanding of how the biomass composition of wingnut varies by location. Samples were collected from individual wingnuts at 7 separate locations: Ames, Iowa; Haysville, Kansas; Columbia, Missouri; Lincoln, Nebraska; Brookings, South Dakota; Madison, Wisconsin; Glencoe, Illinois; and Urbana, Illinois. Two samples were collected from two individuals at each location except for Madison, Wisconsin and Glencoe, Illinois, where samples were collected from only a single tree due to limited availability. Thirty samples were collected from the population grown at the University of Illinois energy farm, the purpose being to establish a chemical composition base line.

## Sample collection

Except for material collected for the compositional baseline, biomass samples were collected in the form of tree limbs, beginning with shoots from the current growing year and going back successively until the fourth growing year. Before samples were cut, samples were aged via location of bud scale scarring on branches and examination of growth rings under magnification. Samples were collected as 30-cm long segments (includes 1- to 4-year-old wood) and placed into plastic bags in coolers with ice for the duration of the collection trip. After the collection trip, the samples were oven dried at 60°C in a forced air drying oven, milled with a Retsch SM 200 cutting mill with a 2-mm screen (Haan, Germany).

After milling, the material was ground to a powder in a SPEX sample prep Geno Grinder 2000 ball mill (Metuchen, NJ, United States). Samples were then sent to the Energy Biosciences Institute Analytical Lab for compositional analysis.

Samples for the compositional baseline were collected from the Urbana EBI farm population from trees that were established in 2010. Samples collected from the farm population consisted of single shoots with an average diameter of 12 mm and comprised of two-year-old wood. Once collected, the shoots were then processed in the same manner as the samples collected from various locations throughout the Midwest. All plants utilized in this study originated from common offspring that were seed propagated from a 1985 Holden Arboretum (Kirtland, OH) seed collection trip to Kwangnung Forest Research Station, Kyong Gi Do, Namyeongju Gun, South Korea, along a stream in a flood plain.

### **Soil sampling**

Soil subsamples were collected at the base, drip line and midway between base and drip line of each tree, and then combined to create a single representative sample per individual tree. Soil samples were collected with a 2.3-cm diameter soil probe (JBK Mfg., Dayton, OH, United States) sampled at a depth of 1 to 20 cm. Samples were then shipped to A & L Great Lakes Laboratories, Inc. for analysis with the following parameters being reported: organic matter, available phosphorus, exchangeable potassium, magnesium, calcium, pH, buffer pH, cation exchange capacity, percent base saturation of cation elements, sulfur, zinc, manganese, iron, copper, boron and particle size. Information on soil profile and soil texture at the sample sites can be found in tables 2 and 3.

### **Composition testing procedures**

Sample preparation followed NREL laboratory analytical procedures (LAP) for compositional analysis (Hames et al., 2008). Wingnut tissue not already dried and ground at Urbana, IL, was ground using a high speed rotor mill (Ultra Centrifugal Mill ZM 200, Retsch) passing a 2 mm sieve and then oven-dried (105 °C for 16 h). In a 5-mL extraction cell, 1 gram of biomass and a pre-weighed microfiber filter (Dionex, Sunnyvale, CA, USA) were extracted with water and ethanol in an accelerated solvent extractor (ASE350, Dionex). The extraction conditions were as follows: temperature 100 °C, 5 min holding time, 3 cycles per solvent, 150% rinse, 60 s nitrogen purge (Hames et al., 2008). The biomass and filter were quantitatively transferred into pre-weighed aluminum pans and dried at 105°C for 16 h and cooled in a desiccator for 30 min. The difference between starting mass and mass after extraction determined the amount of extractives.

Determination of structural carbohydrates and lignin in the biomass followed NREL LAP (Sluiter et al., 2011). Extracted wingnut tissue was ball-milled for 5 min using a canister ball-mill (model 8200

tissue pulverizer, Kinetic Laboratory Equipment Company, Visalia, CA) and the biomass was then oven-dried (105°C for 16 h). For total sugar analysis, 50 mg of ball-milled biomass were incubated at room temperature with 0.5 mL of 72% (w/w) sulfuric acid in a modified Hungate vial capped with a rubber stopper and vortexed every 15 min. After 1 hr, 14 mL of deionized water was added, and the mixture was autoclaved for 60 min (Sluiter et al., 2011). For hemicellulose analysis, 50 mg of ball-milled biomass was incubated with 14.5 mL of 4% (w/w) sulfuric acid for 10 min, and the mixture was autoclaved for 60 min. A sugar recovery standard containing glucose, xylose and arabinose and the same sulfuric acid concentration was prepared in a similar way and co-autoclaved with the samples. After cooling to room temperature, the mixture was vigorously shaken and was kept in the refrigerator overnight, then 2 mL of the clear supernatant was filtered (0.45 µm, PES) and used for HPLC analysis.

Determination of Klason lignin in the biomass followed NREL LAP (Sluiter et al., 2005) with slight modification. The precipitated solids were re-suspended by vortexing, and the suspension was filtered through a glass micro filter. The filter was extensively rinsed with deionized water and dried at 105 °C overnight; the weight (m1) was determined after cooling in a desiccator for 30 min. The filter and solids were then incubated at 575 °C (ramp: 105 °C for 10 min, 200 °C for 10 min, 300 °C for 30 min, 575 °C for 3 h, cooling to 105 °C); the weight (m2) was determined after cooling in a desiccator for 30 min. The difference m1 - m2 resulted in Klason lignin (ash corrected).

For ash determination, 50 -100 mg of biomass were incubated on a pre-weighed aluminum pan at 575°C (ramp: 105°C for 10 min, 200°C for 10 min, 300°C for 30 min, 575°C for 3 h, cooling to 105°C); and the weight was determined after cooling in desiccator for 30 min (Sluiter et al., 2005).

Samples were analyzed after acid hydrolysis for monosaccharides and acetate at 50°C by HPLC on an organic acid separation column (Aminex HPX-87H, 300 x 7.8 mm, Bio Rad, Richmond, CA) on a 1200 series HPLC with a refractive index detector (Agilent Technologies, Santa Clara, CA). Elution was performed with 0.005 M sulfuric acid at a flow rate of 0.6 mL/min. For calibration, solutions of reference compounds in the range of 0.01 - 10 mg were prepared. The following conversions were applied: [% glucan = % glucose \* 0.9], [% xylan = % xylose \* 0.88], [% arabinan = % arabinose \* 0.88], [% cellulose = % total glucan - % hemicellulose glucan].

## Results

### Farm population

Samples of wingnut collected from the University of Illinois energy farm, Urbana, IL, in January of 2012 were compared with values reported in the literature for other crops currently under investigation as potential short rotation woody crops for feedstock purposes. Data was collected on

monosaccharide content, ethanol soluble extractives, and structural composition. Monosaccharide content comparisons showed that wingnut has the lowest glucan content of the comparison group of species at 28.9% (Table 4). Given that glucan is the main polysaccharide of cellulose, this matches the wingnut's comparatively low levels of cellulose (Table 5). Wingnut samples yielded above average levels of arabinan (1.1%) and average levels of xylan (15%). Since arabinan and xylan are the major components of hemicellulose, these findings correlate with the somewhat average 17% dry mass of hemicellulose (Table 5). The 17% dry mass composition of hemicellulose is on par with other species except for *Salix alba* and *Salix viminalis* which have significantly higher hemicellulose levels of 26.6 and 25.6%, respectively (Stolarski et al., 2011).

Wingnut has a much higher percentage of extractives than any of the other species used in the comparison group (Table 6). The high concentration of extractives found in the wingnut samples may account for the unusually low levels of cellulose, as extractives are counted as a portion of total drymass, therefore lowering the total percentage of drymass that could be attributed to cellulose (Table 5).

When comparing biomass compositional components to other possible SRWC species, wingnut is unfortunately unimpressive with a cellulose level of 25.2%, far below the average range for most hardwoods of 40-50% (Rowel, 1984). Wingnut does however share similar levels of hemicellulose, lignin and ash (Table 5). It should be noted that wingnut has the second lowest lignin levels only slightly higher than that of *Salix viminalis* (Stolarski et al., 2011). Considering that carbohydrates containing the five and six carbon sugars necessary for fermentation are found primarily in cellulose and hemicellulose, the extremely low levels of cellulose make wingnut a poor candidate for any type of liquid transportation fuel relying on the use of sugars as a fuel source regardless of the conversion pathway.

When considering the applicability of the crop for the use as a biofuel feedstock, the ratio of cellulose to lignin should be considered. Having already established that Chinese wingnut has below average levels of cellulose when compared to other hardwoods, with most hardwoods having between 40 – 50% cellulose content (Rowell, 2005), it would also be illustrative to examine the ratio of cellulose to lignin. The lignin content of Chinese wingnut at 22.1% of dry mass is comparable to other commonly considered SRWC's, however at a cellulose to lignin ratio of 1.14 Chinese wingnut ranks lowest in overall cellulose content and cellulose to lignin ratio of our comparison species. Comparable species *Robinia pseudoacacia*, *Salix viminalis* (Tur), and *Populus deltoides* Stoneville 66 have cellulose to lignin ratios of

1.41, 1.97, and 1.65 respectively. The low cellulose to lignin ratio again illustrates the ill-suited nature of this crop as a biomass feedstock.

### **Biomass via year**

It should be noted that the biomass estimation models were developed in Canada where growing seasons are shortened and the models were calibrated for trees grown in forested settings. Given that the wingnuts used in this study were grown in a plantation setting, resulting in relatively open conditions, it can be assumed that the biomass estimations reported here are underestimations of the actual biomass produced in open conditions. Despite these shortcomings, the models are useful in providing relative estimates of the trees' total above-ground dry biomass and providing a reasonable estimate of actual biomass.

The University of Missouri NC7 trail site in New Franklin Missouri had three wingnut trees along with other species as part of an ornamentals evaluation trial. Their trees were planted with a spacing of 4.5 m within the row and 6 m between rows on level ground. Trees were at one point drip irrigated and the plantation was mowed regularly. Wingnuts at this location typify wingnuts found at other locations with multiple lateral branches forming low in the crown, below breast height (1.3m), with obtuse branch angles giving the trees a low spreading appearance. It should be noted however, that proper pruning can produce a single straight trunk and a more formal ornamental appearance. The two trees from which samples were collected were both 12 m tall, with the first tree having 4 branches below breast height, measuring 31.5, 30.7, 9.9, and 14.9 cm in circumference (Table 7). The second tree also had 4 stems below breast height, measuring 16.5, 28.7, 14.5, and 27.9 cm in circumference.

Wingnuts located at the Kansas State University Horticultural Center were planted 3 m apart within the row and 7.6 m apart between rows, resulting in a degree of canopy closure produced by surrounding trees. The plantation was situated on well-drained soil with no slope, with surrounding turf mowed regularly, and no irrigation provided. Tree one had a DBH of 15.7 cm and was 8.1 m in height, and tree two was 6.3 m in height and had two branches below breast height, measuring 30.3 and 23.9 cm. Trees one and two had an estimated above ground dry biomass of 69 and 54 kg, respectively, far below the average biomass of the other trees in this comparison.

Wingnuts located at the Nebraska Statewide Arboretum, located in Lincoln, Nebraska, were grown in an open setting with no light competition, a slight slope with surrounding turf mowed regularly, and trees were not irrigated. The Nebraska trees had an estimated above ground biomass of 251 and 63 kg, well below the average.

Wingnuts located at the Iowa State University horticulture research facility located in Ames, Iowa were spaced, along with other species undergoing landscape trials, 1.8 m within the row, with rows 7.6 m apart. Trees were located in the center of a 4.2-m herbicide-treated strip to control weeds and grasses, and the plantation had a slight slope. Tree one had two branches below breast height and had an estimated above ground dry biomass of 387 kg, similar to the average for all trees in this study. Tree two had an estimated dry biomass of 476 kg.

South Dakota State University horticultural research station located in Brookings, South Dakota had two trees planted along with other species, with 2.1 m spacing within rows and 4.5 m between rows. Trees were grown in relatively open conditions with surrounding species being smaller and thus providing little light competition, and surrounding turf was mowed regularly. Tree one had three stems below breast height and tree two had three stems below breast height with relatively low estimated above ground dry biomass of 133 kg and 116 kg, respectively.

The single wingnut located at the University of Wisconsin Arboretum located in Madison, Wisconsin was open planted with no light competition. The tree measured 29.2 cm in circumference at breast height and had a height of 12 m with an estimated above ground biomass of 307 kg. The tree had been pruned regularly and therefore had a more upright form with a single trunk making for a more attractive looking specimen.

The single wingnut located at the Chicago Botanical Gardens located in Glencoe, Illinois was planted on open ground on top of a berm with no light competition and measured 18.8 cm in circumference and 5 m in height, with an estimated aboveground dry biomass of 94 kg. The tree had poor form, showed signs of physical damage and disease, possibly contributing to its poor growth. Its location on the top of a berm, a very dry location, also likely contributed to its small size.

The wingnuts located at the University of Illinois Landscape Horticulture Research Center in Urbana, IL were grown in an arboretum setting. Tree one was located in a small opening with some light competition, whereas tree two was planted adjacent to a stand of trees that created a significant degree of light competition. Tree one had a single trunk at DBH measuring 56.9 cm with a height of 11 m. Tree two has a single trunk at DBH measuring 53.3 cm and a height of 12 m. Trees one and two had estimated above ground dry biomass of 1341 and 1174 kg, respectively.

### **Compositional Analysis**

The samples collected were separated by years of growth ranging from last year's growth (yr. 1) to the 4 years prior (yr. 4). Cellulose composition increased with age (Fig. 19) of growth, increasing from 17.6 to 26.9% in years 1 to 4. Hemicellulose appeared to increase from 15.0 to 16.3% in years 1 to 4,



however the differences were not significant. Lignin content appeared decrease from 26.6 in year 1 to 24.6% in year 4 with differences again not significant. The ash content also appeared to decrease with increasing age, from 3.37 – 1.99%. Extractives decrease from 18.8 to 12.5% from years 1 to 3, but were unchanged in year 4. The level of extractives reported here is much higher than those of other hardwood species. Given that bark extractives are known to have medicinal and pesticide activities it may be true that this correlates with the high levels of extractives found in the biomass. The acetyl composition increased slightly from 2.5 – 3.12% over years 1 to 4. These patterns of increasing cellulose, upward trending levels of hemicellulose, downward trending of lignin and ash and increases in extractives and acetyl could be due to the ratio of bark to wood decreasing with age, with bark containing higher concentrations of lignin, extractives and ash, or they may be due to some other less understood factor.

If the supposition that the difference in composition from year 1 to year 4 is driven largely by the decreasing ratio of bark and young tissue to older wood which is no longer conductive, then it is fair to assume that the apparent lower rate of change between years 3 and 4 is due to smaller changes in the ratio between these tissue types. If the above mentioned rate of change diminishes as the tree ages, it might then be reasonable to assume that samples taken from older sections of the tree having much larger diameters may have similar compositional values to that of the year 4 samples.

Composition values reported for samples collected from the energy farm population (two-year-old stems) are consistent with values from 2<sup>nd</sup> year samples obtained from similarly aged stems taken from the range of trees around the Midwest, table 8. Of all the values compared, lignin content varies the most with the lignin content of samples collected from various locations within the Midwest being 3.2 percentage points higher than that of the farm population. The general consistency of the remaining compositional components is not surprising as material collected at the farm had an average diameter of 1.25 cm consistent with that of 2<sup>nd</sup> year material collected elsewhere.

Chinese wingnut was originally selected for examination due to a reputation for having a fast growth rate in the Urbana NC-7 ornamental evaluation trials. The population of wingnuts located at the University of Illinois energy farm did not have an impressive growth rate (authors unpublished visual observations), when compared to other species such as poplar, willow and black locust grown at the same site. Simple visual examination made it clear that for the particular location and environmental conditions, Chinese wingnut did not possess an acceptable growth rate for biomass production. Various sources including a US forest service fact sheet (U.S. Forest service, 1994) indicate that the species does best when located near a river or stream. The wingnuts located at the University of Illinois energy farm

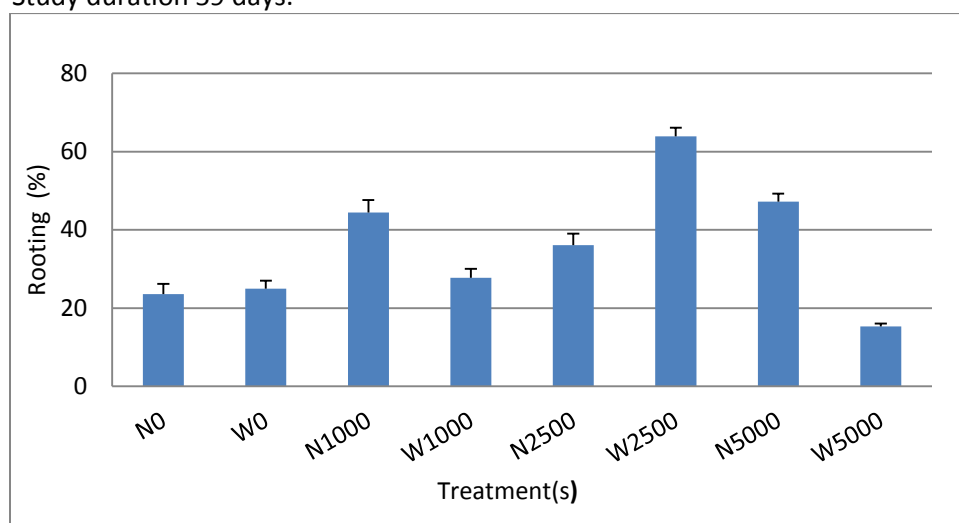
were not located near a source of surface water, and had endured two seasons of drought this possibly explains that unexpectedly low growth rate.

Initially the biomass via year study was intended to test how biomass changed from year to year to determine if significant changes could be found in biomass characteristics. Roughly 25% of the samples were lost at some point after collection and before compositional analysis, the remaining sample population was too small to for statistical analysis. If this study were repeated a larger number of samples would have been collected to allow for potential losses and analysis.

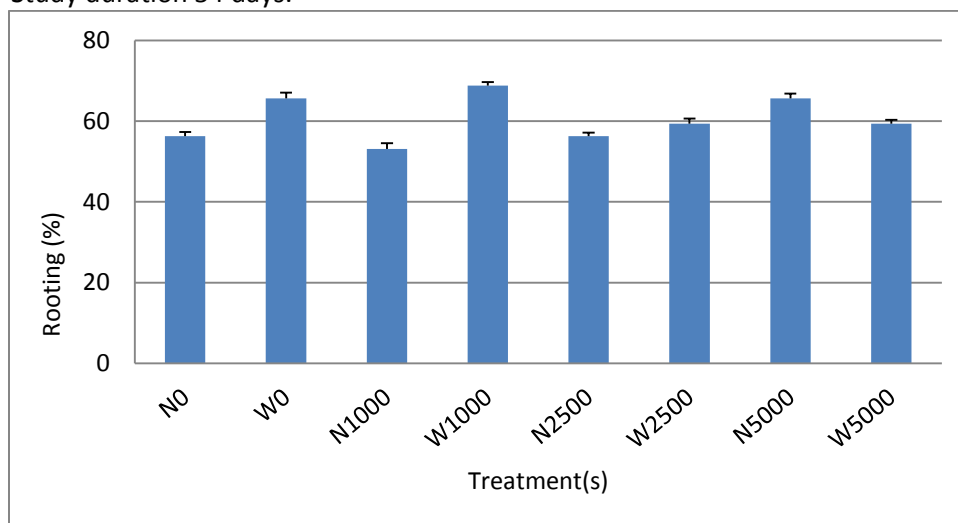
In conclusion, we see that cellulose and hemicellulose values increase from growing years one to four in contrast to compositional content of lignin, ash, and extractibles with the level of acetyl remaining fairly constant. When comparing second year material to that of material collected at the energy farm it is clear that the chemical composition does not vary widely given location and slightly different climate.

# Figures

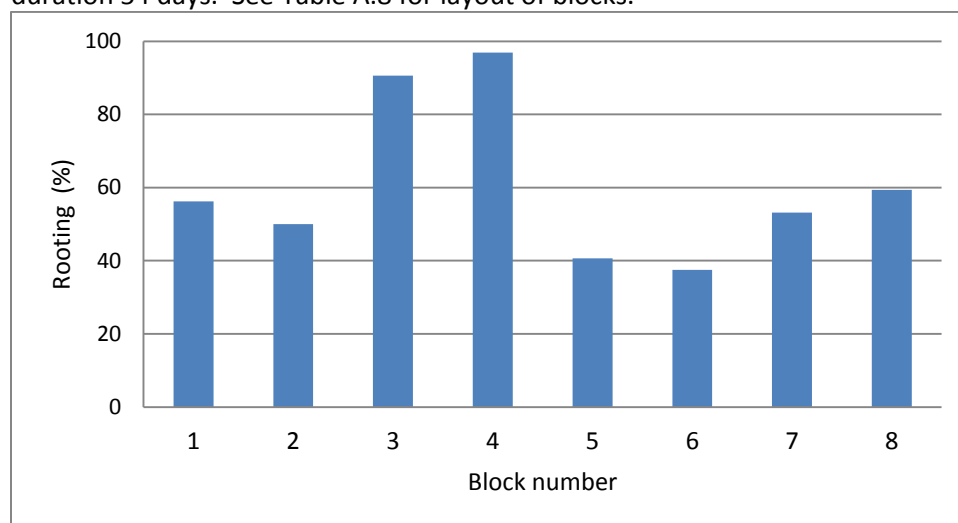
**Fig. 1. Effect of wounding and IBA on the rooting percentage of *Pterocarya stenoptera* first trial hardwood terminal cuttings.** N= Non wounded, W= cuttings wounded by removal of a single 2 to 3cm long sliver of bark from the basal end of the cutting exposing the cambium. IBA concentrations of 0, 1000, 2500, and 5000 ppm applied as a 5-second quick dip in 25% ethanol:water (v:v). Study duration 59 days.



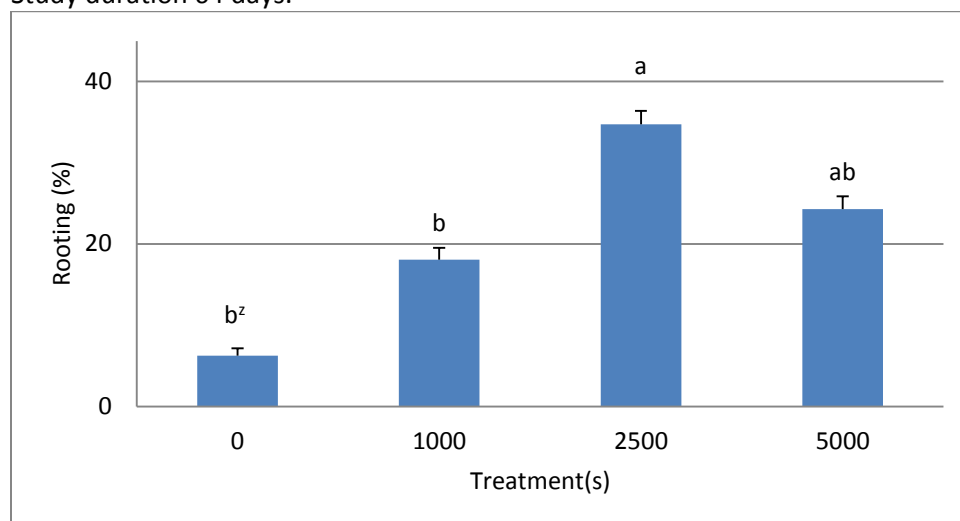
**Fig. 2. Effect of wounding and IBA on the rooting percentage of *Pterocarya stenoptera* second trial hardwood terminal cuttings .** N= Non wounded, W= cuttings wounded by removal of a single 2 to 3cm long sliver of bark from the basal end of the cutting exposing the cambium. IBA concentrations of 0, 1000, 2500, and 5000 ppm applied as a 5-second quick dip in 25% ethanol:water (v:v). Study duration 54 days.



**Fig. 3. Effects of mist bench location (blocking) on the rooting percentage of second trial hardwood terminal cuttings of *Pterocarya stenoptera* treated with 0 to 5000 ppm IBA applied with liquid quick dip and wounded and non-wounded treatment.** Cuttings wounded by removal of a single 2 to 3cm long sliver of bark from the basal end of the cutting exposing the cambium. Rooting percentages are averaged over all IBA and wounding treatments. Study duration 54 days. See Table A.8 for layout of blocks.

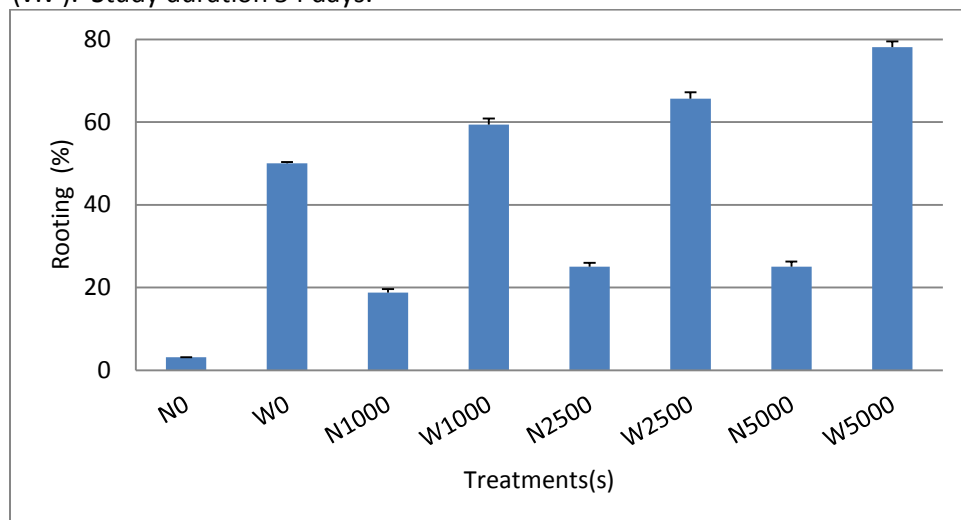


**Fig. 4. Effect of IBA on the rooting percentage of *Pterocarya stenoptera* first trial hardwood sub-terminal cuttings.** IBA concentrations of 0, 1000, 2500, and 5000 ppm applied as a 5-second quick dip in 25% ethanol:water (v:v). Study duration 64 days.

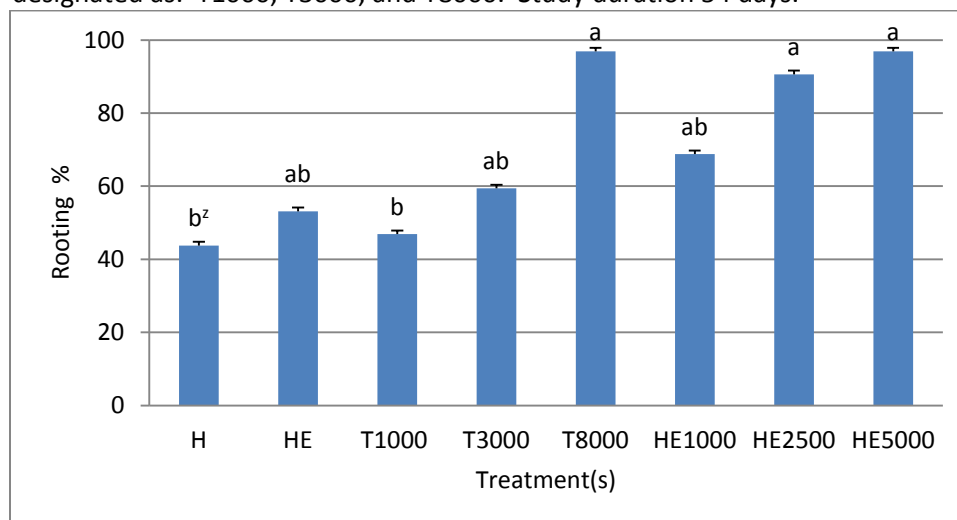


<sup>2</sup>Grouping significant at 0.05

**Fig. 5. Effect of wounding and IBA on the rooting percentage of *Pterocarya stenoptera* second trial hardwood sub-terminal cuttings.** N= Non wounded, W= cuttings wounded by removal of a single 2 to 3cm long sliver of bark from the basal end of the cutting exposing the cambium. IBA concentrations of 0, 1000, 2500, and 5000 ppm applied as a 5-second quick dip in 25% ethanol:water (v:v). Study duration 54 days.

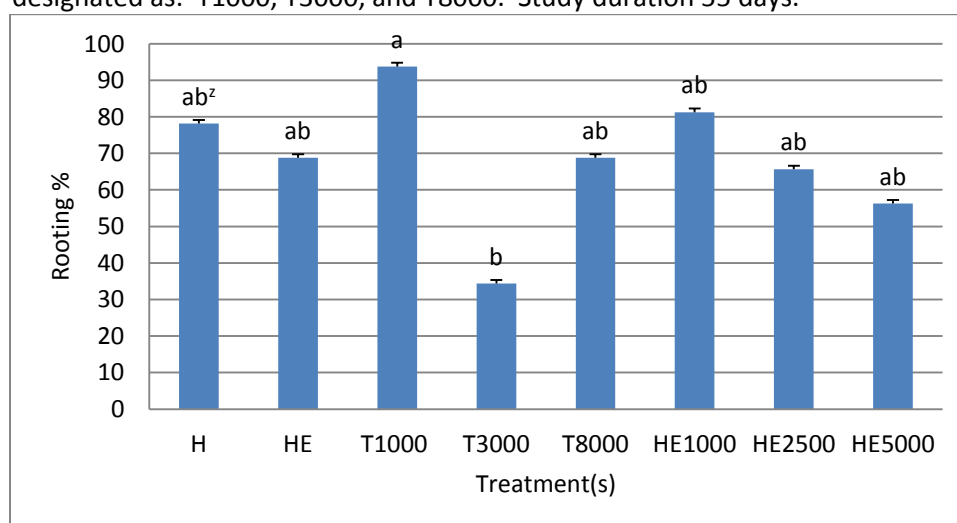


**Fig. 6. Effect of IBA on the rooting percentage of *Pterocarya stenoptera* first trial semi-hardwood terminal cuttings.** Treatments applied with water and ethanol include, water alone (H), 25% ethanol in water (HE) and mixtures of 25% ethanol in water with IBA concentrations ranging from 1000 to 5000 ppm: HE1000, HE2500, and HE5000, applied as a 5-second quick dip. Application of IBA with a talcum powder carrier (Hormodin) with 1000 to 8000 ppm are designated as: T1000, T3000, and T8000. Study duration 54 days.



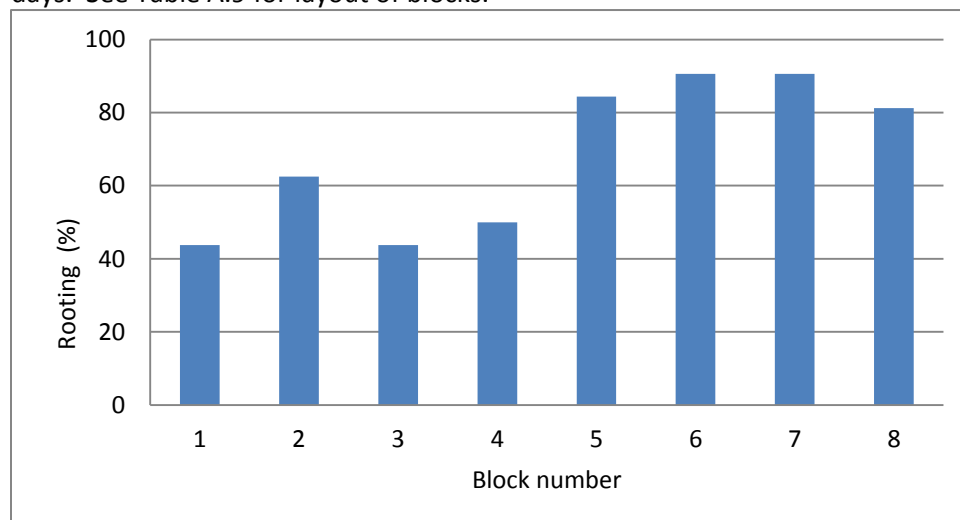
<sup>z</sup>Grouping significant at 0.05

**Fig. 7. Effect of IBA on the rooting percentage of *Pterocarya stenoptera* second trial semi-hardwood terminal cuttings.** Treatments applied with water and ethanol include, water alone (H), 25% ethanol in water (HE) and mixtures of 25% ethanol in water with IBA concentrations ranging from 1000 to 5000 ppm: HE1000, HE2500, and HE5000, applied as a 5-second quick dip. Application of IBA with a talcum powder carrier (Hormodin) with 1000 to 8000 ppm are designated as: T1000, T3000, and T8000. Study duration 55 days.

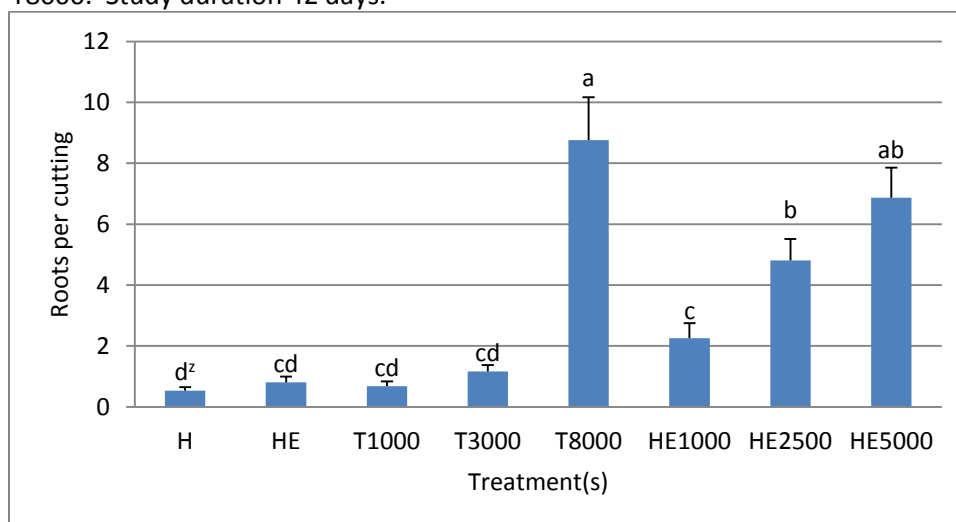


<sup>z</sup>Grouping significant at 0.05

**Fig. 8. Effects of mist bench location (blocking) on the rooting percentage of second trial semi-hardwood terminal cuttings of *Pterocarya stenoptera* treated with 0 to 8000 ppm of IBA applied with liquid or talc-based carriers.** Rooting percentages are averaged over all IBA treatments. Study duration 55 days. See Table A.9 for layout of blocks.

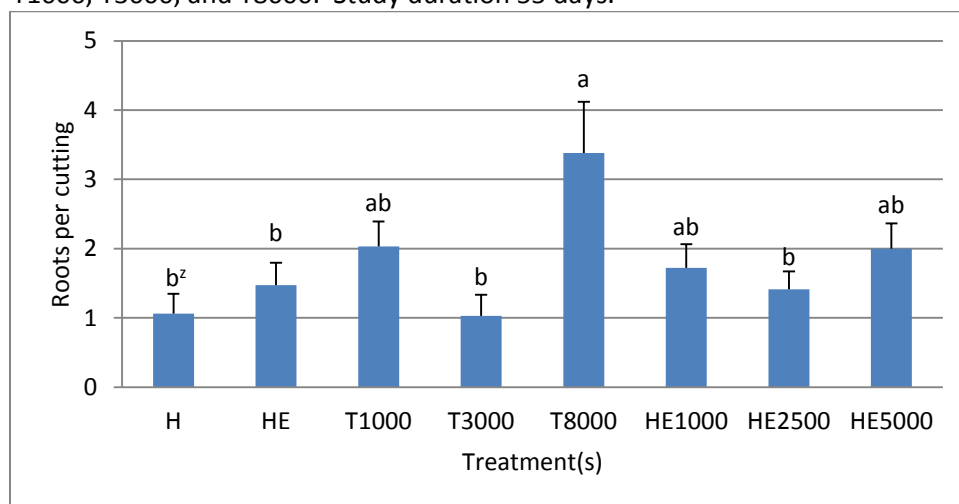


**Fig. 9. The effect of IBA and carrier on the average number of roots produced per cutting (+ SE) of first trial semi-hardwood terminal cuttings of *Pterocarya stenoptera*.** Treatments applied with water and ethanol include, water alone (H), 25% ethanol in water (HE) and mixtures of 25% ethanol in water with IBA concentrations ranging from 1000 to 5000 ppm: HE1000, HE2500, and HE5000, applied as a 5-second quick dip. Application of IBA with a talcum powder carrier (Hormodin) with 1000 to 8000 ppm are designated as: T1000, T3000, and T8000. Study duration 42 days.



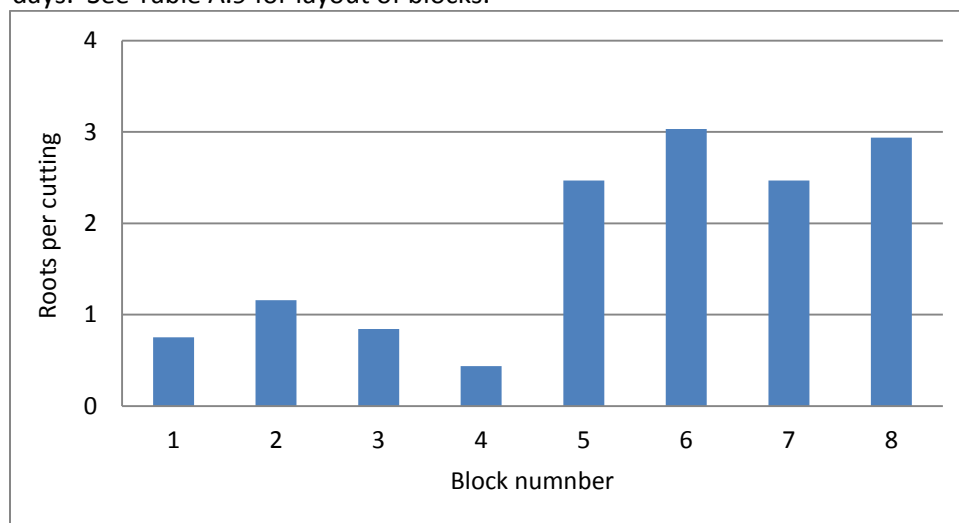
<sup>z</sup>Grouping significant at 0.05

**Fig. 10. The effect of IBA and carrier on the average number of roots produced per cutting (+ SE) of second trial semi-hardwood terminal cuttings of *Pterocarya stenoptera*.** Treatments applied with water and ethanol include, water alone (H), 25% ethanol in water (HE) and mixtures of 25% ethanol in water with IBA concentrations ranging from 1000 to 5000 ppm: HE1000, HE2500, and HE5000, applied as a 5-second quick dip. Application of IBA with a talcum powder carrier (Hormodin) with 1000 to 8000 ppm are designated as: T1000, T3000, and T8000. Study duration 55 days.



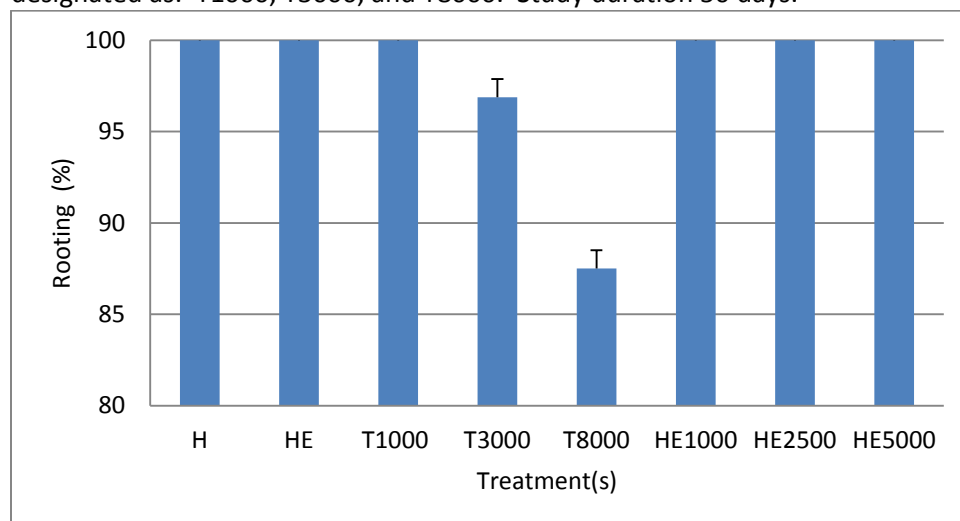
<sup>z</sup>Grouping significant at 0.05

**Fig. 11. Effects of mist bench location (blocking) on the rooting percentage of second trial semi-hardwood terminal cuttings of *Pterocarya stenoptera* treated with 0 to 8000 ppm of IBA applied with liquid or talc-based carriers.** Rooting percentages are averaged over all IBA treatments. Study duration 55 days. See Table A.9 for layout of blocks.

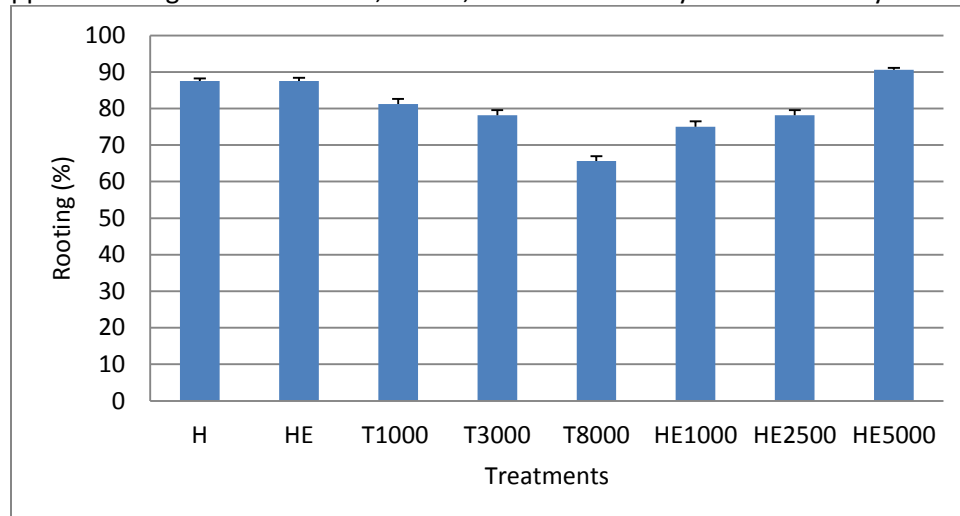




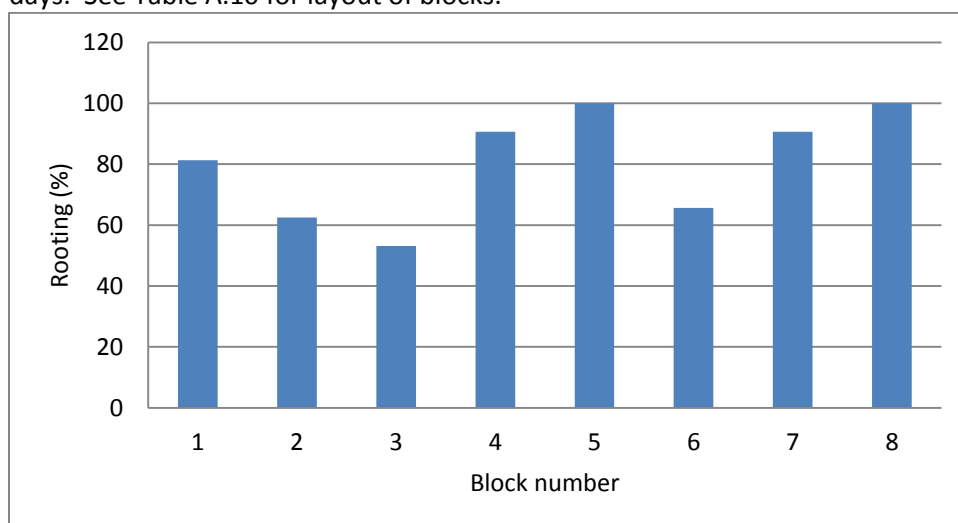
**Fig. 12. Effect of IBA on the rooting percentage of *Pterocarya stenoptera* first trial semi-hardwood sub-terminal cuttings .** Treatments applied with water and ethanol include, water alone (H), 25% ethanol in water (HE) and mixtures of 25% ethanol in water with IBA concentrations ranging from 1000 to 5000 ppm: HE1000, HE2500, and HE5000, applied as a 5-second quick dip. Application of IBA with a talcum powder carrier (Hormodin) with 1000 to 8000 ppm are designated as: T1000, T3000, and T8000. Study duration 50 days.



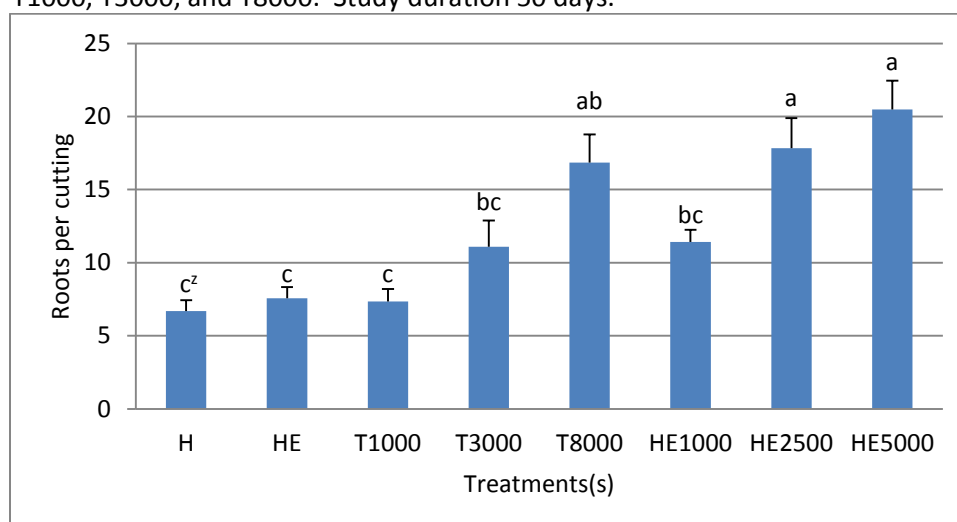
**Fig. 13. Effect of IBA on the rooting percentage of *Pterocarya stenoptera* second trial semi-hardwood sub-terminal cuttings .** Treatments applied with water and ethanol include, water alone (H), 25% ethanol in water (HE) and mixtures of 25% ethanol in water with IBA concentrations ranging from 1000 to 5000 ppm: HE1000, HE2500, and HE5000, applied as a 5-second quick dip. Application of IBA with a talcum powder carrier (Hormodin) with 1000 to 8000 ppm are designated as: T1000, T3000, and T8000. Study duration 55 days.



**Fig. 14. Effects of mist bench location (blocking) on the rooting percentage of second trial semi-hardwood sub-terminal cuttings of *Pterocarya stenoptera* treated with 0 to 8000 ppm of IBA applied with liquid or talc-based carriers. Rooting percentages are averaged over all IBA treatments. Study duration 55 days. See Table A.10 for layout of blocks.**

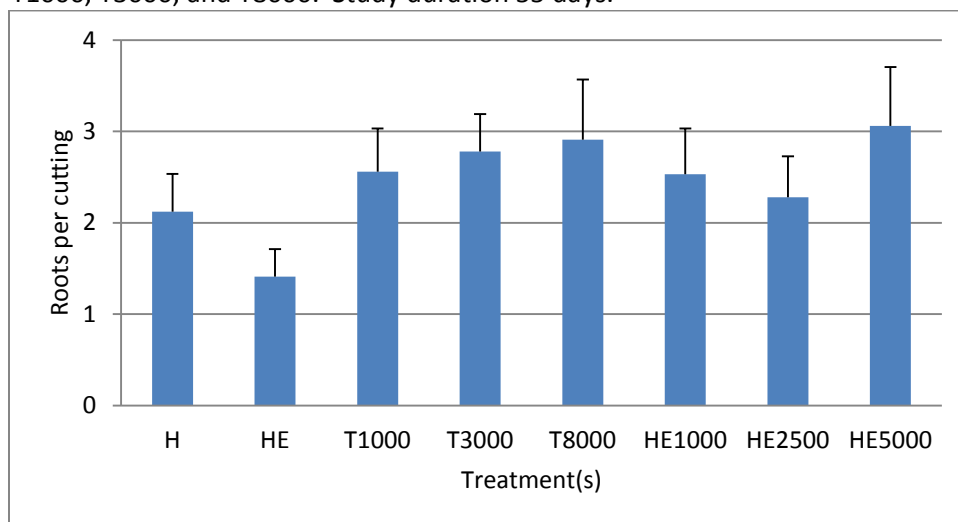


**Fig. 15. The effect of IBA and carrier on the average number of roots produced per cutting (+ SE) of first trial semi-hardwood sub-terminal cuttings of *Pterocarya stenoptera*. Treatments applied with water and ethanol include, water alone (H), 25% ethanol in water (HE) and mixtures of 25% ethanol in water with IBA concentrations ranging from 1000 to 5000 ppm: HE1000, HE2500, and HE5000, applied as a 5-second quick dip. Application of IBA with a talcum powder carrier (Hormodin) with 1000 to 8000 ppm are designated as: T1000, T3000, and T8000. Study duration 50 days.**

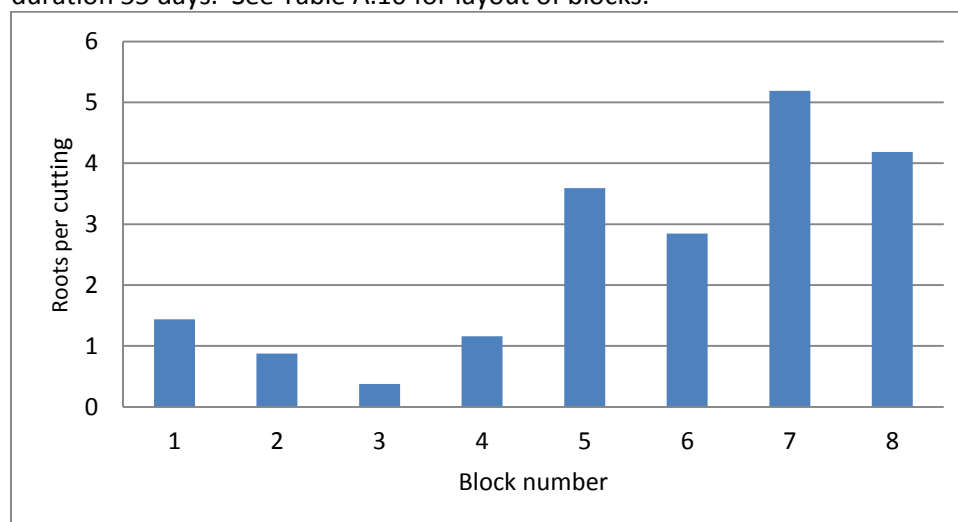


<sup>z</sup>Grouping significant at 0.05

**Fig. 16. The effect of IBA and carrier on the average number of roots produced per cutting (+ SE) in second trial semi-hardwood subterminal cuttings of *Pterocarya stenoptera*.** Treatments applied with water and ethanol include, water alone (H), 25% ethanol in water (HE) and mixtures of 25% ethanol in water with IBA concentrations ranging from 1000 to 5000 ppm: HE1000, HE2500, and HE5000, applied as a 5-second quick dip. Application of IBA with a talcum powder carrier (Hormodin) with 1000 to 8000 ppm are designated as: T1000, T3000, and T8000. Study duration 55 days.

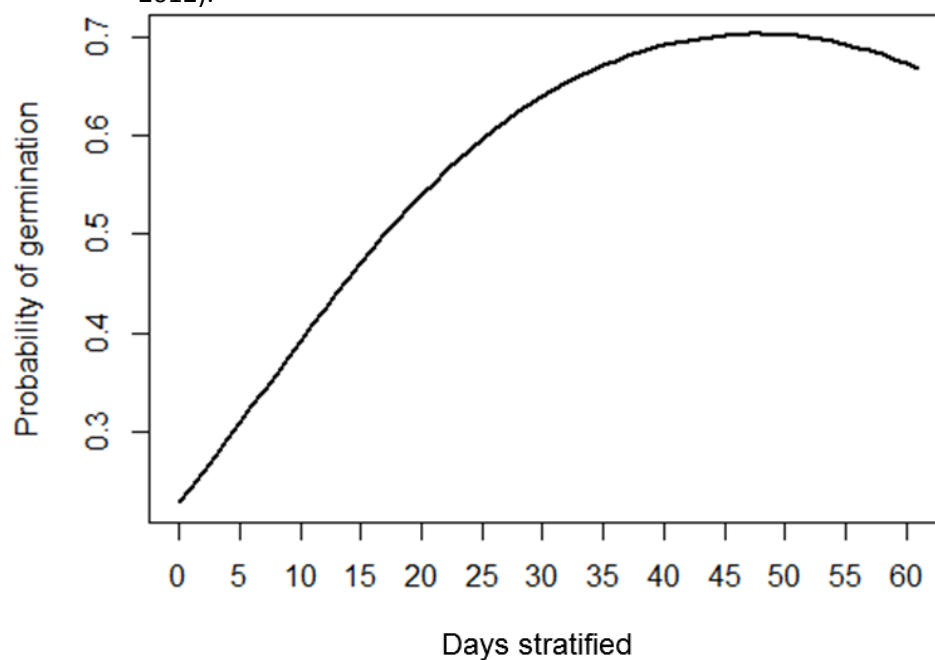


**Fig. 17. Effects of mist bench location (blocking) on the rooting percentage of second trial semi-hardwood sub-terminal cuttings of *Pterocarya stenoptera* treated with 0 to 8000 ppm of IBA applied with liquid or talc-based carriers (July, 2012).** Rooting percentages are averaged over all IBA treatments. Study duration 55 days. See Table A.10 for layout of blocks.

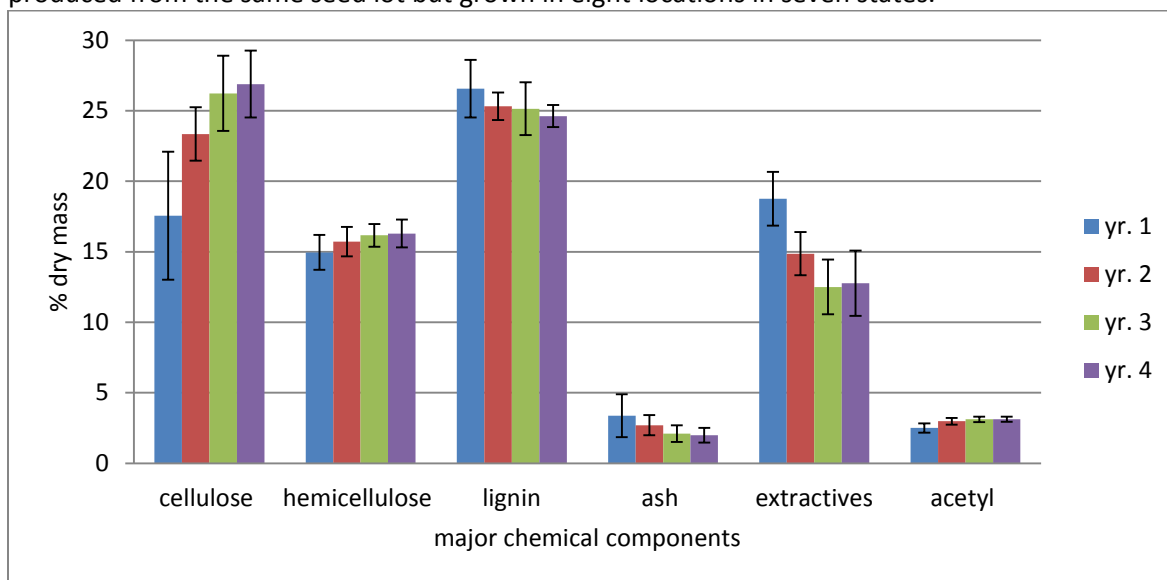


**Fig. 18. Germination probability of a single *Pterocarya stenoptera* seed as affected by days of moist stratification at 4.5°C.**

Probability of germination is based on results from both first (February, 2011) and second (September, 2012) trials of seed germination data. Germination probabilities generated by logistic regression model conducted in R using GLM function (R Core Team, 2012).



**Fig. 19. Percentage of major chemical components of 1- to 4-year-old stems of *Pterocarya stenoptera*, reported as percentage of dry shoot mass.** Wood samples were obtained from trees produced from the same seed lot but grown in eight locations in seven states.



**Table 1. Germination rates as a result of days of stratification for seeds of *Pterocarya stenoptera*.** Seeds stratified in moist sand at 4.5 C° for first trial (February, 2011) and second trial (September, 2012). 30 seeds planted in five day intervals.

First trial			Second trial		
Days	Count	% germination	Days	Count	% germination
0	7	23.3	0	7	23.3
5	17	56.7	5	12	40.0
10	18	60.0	10	9	30.0
15	22	73.3	15	14	46.7
20	22	73.3	20	15	50.0
25	22	73.3	25	15	50.0
30	27	90.0	30	22	73.3
35	19	63.3	35	22	73.3
40	24	80.0	40	15	50.0
45	23	76.7	45	16	53.3
50	24	80.0	50	12	40.0
55	26	86.7	55	19	63.3
60	23	76.7	60	16	53.3

**Table 2. Soil profile of various sample locations of *Pterocarya stenoptera***

	Organic matter	Bray-1 equiv ppm-p	K ppm	Mg ppm	Ca ppm	Soil pH	Buffer pH	CEC meq/100g	%K	%Mg	%Ca	%H	S ppm	Zn ppm	Mn ppm	Fe ppm	Cu ppm	B ppm
Wisconsin	4.2	10	94	205	1050	6	6.8	9.6	2.5	17.8	54.7	25	5	5.4	84	37	1.2	0.4
Iowa	23	13	86	300	1600	6.6	6.9	11.9	1.8	21	67.1	10.1	5	3.1	58	26	1.1	0.4
South Dakota 1	2.3	23	110	465	2250	7.5		15.4	1.8	25.2	73		5	1.8	51	1	1.5	1
South Dakota 2	2.8	25	113	440	3200	7.7		20	1.5	18.4	80.2		5	2.2	47	1	1.3	0.8
Urbana 1	3.4	7	274	380	1250	6.5	6.9	11.3	6.2	28	55.2	10.6	7	6	36	13	1.3	0.8
Urbana 2	3.5	14	290	30	1150	6.2	6.8	11.7	6.3	24.2	49	20.5	7	2.9	31	21	1.2	0.6
Nebraska 1	3.5	17	341	605	2300	6.1	6.7	21	4.2	24	54.7	17.1	7	1.7	34	27	1.4	0.6
Nebraska 2	2.5	20	391	795	2450	6.3	6.7	23.5	4.3	28.2	52.2	15.3	6	1.7	41	25	1.7	0.5
Kansas 1	1.7	2	146	270	1850	6.7	6.9	13.1	2.9	17.2	70.7	9.2	6	1.5	32	5	1.4	0.6
Kansas 2	2.1	1	158	295	2200	6.6	6.9	15.1	2.7	16.3	73	8	7	1.3	32	7	1.6	0.7
Missouri 1	1.7	13	124	190	1050	5.8	6.8	9.6	3.3	16.6	55	25.1	6	1.8	47	27	0.9	0.2
Missouri 2	1.8	12	158	125	1050	6.3	6.9	7.9	5.1	13.2	66.5	66.5	5	2.5	63	31	0.9	0.2
Chicago	7.3	28	201	595	3250	7.6		21.7	2.4	22.8	74.8		16	7.9	31	73	3.6	2.7
	Phosphorus					pH			% Cation saturation									

**Table 3. Soil texture of various sample locations of *Pterocarya stenoptera***

Location	Soil texture class	%Sand	%Silt	%Clay
Wisconsin	Silt loam	19	58	23
Iowa	Loam	45	32	23
South Dakota 1	Clay loam	45	26	29
South Dakota 2	Clay loam	41	30	29
Urbana 1	Clay loam	29	44	27
Urbana 2	Silt loam	25	50	25
Nebraska 1	Silty clay	11	46	43
Nebraska 2	Silty clay	9	44	47
Kansas 1	Sandy clay loam	51	22	27
Kansas 2	Sandy clay loam	47	22	31
Missouri 1	Silt loam	11	64	25
Missouri 2	Silt loam	15	66	19
Chicago	Clay loam	33	38	29

**Table 4. Content of monosaccharide, and acetyl (% dry mass) of 1 to 3-year-old shoots of *Pterocarya stenoptera* grown in Urbana, Illinois, compared to values found in the literature for other common short rotation woody crops.**

Species	Arabinan	Xylan	Glucan	Acetyl
<i>Eucalyptus saligna</i> <sup>z</sup>	0.3	10.4	48.1	
<i>Platanus occidentalis</i> <sup>z</sup>	0.5	14.4	38.6	
<i>Populus deltoides</i> x <i>nigra</i> DN34 <sup>y</sup>	0.8	13.4	41.1	
<i>Populus deltoides</i> (Stoneville 66) <sup>x</sup>	0.6	13.4	42.2	
<i>Pterocarya stenoptera</i>	1.1	15	28.9	4.7
<i>Robinia pseudoacacia</i> <sup>z</sup>	0.8	15.4	42.1	
<i>Salix miyabeana</i> <sup>w</sup>	0.7	20.9	41.9	4.6
<i>Salix purpurea</i> <sup>w</sup>	0.5	22.5	39.7	4.7

<sup>z</sup> Adam et al., 1994

<sup>y</sup> Sannigrahi et al., 2010

<sup>x</sup> Agblevor et al., 1992

<sup>w</sup> Stolarski et al., 2011

**Table 5. Major chemical constituents as percentage of dry shoot mass<sup>z</sup> of *Pterocarya stenoptera* as compared to other common short rotation woody crops found in the literature.**

Species	Cellulose	Hemicellulose	Lignin	Ash
<i>Eucalyptus saligna</i> <sup>y</sup>	48.1	12.7	26.9	1.2
<i>Platanus occidentalis</i> <sup>y</sup>	39.6	17.8	24.1	1.2
<i>Populus deltoides</i> Stoneville 66 <sup>y</sup>	42.2	16.6	25.6	1
<i>Populus deltoides</i> x <i>nigra</i> DN34 <sup>w</sup>	41.1	17.0	24.3	1.6
<i>Pterocarya stenoptera</i>	25.2	17.9	22.1	1.7
<i>Robinia pseudoacacia</i> <sup>y</sup>	40.4	17.6	28.6	2.1
<i>Salix alba</i> (Duotur) <sup>v</sup>	41.7	26.7	24.5	
<i>Salix viminalis</i> (Tur) <sup>v</sup>	43.1	25.6	21.9	

<sup>z</sup> percentage of drymass reported as percentage of non-extracted biomass

<sup>y</sup> Adam et al., 1994

<sup>x</sup> Sannigrahi et al., 2010

<sup>w</sup> Agblevor et al., 1992

<sup>v</sup> Stolarski et al., 2011



**Table 6. Extractives as percentage of dry shoot mass of *Pterocarya stenoptera* as compared to other common short rotation woody crops found in the literature.**

Species	Extractives (%)	Extraction method
<i>Eucalyptus saligna</i> <sup>z</sup>	4.15	ethanol
<i>Platanus occidentalis</i> <sup>z</sup>	5.49	ethanol
<i>Populus deltoides</i> (Stoneville 66) <sup>y</sup>	2.4	ethanol
<i>Populus deltoides</i> x <i>nigra</i> (DN34) <sup>x</sup>	6.54	ethanol
<i>Pterocarya stenoptera</i>	14.3	ethanol
<i>Robinia pseudoacacia</i> <sup>z</sup>	3.87	ethanol
<i>Salix alba</i> (Duotur) <sup>w</sup>	2.67	ethanol-benzene
<i>Salix viminalis</i> (Tur) <sup>w</sup>	2.68	ethanol-benzene

<sup>z</sup> Adam et al., 1994

<sup>y</sup> Sannigrahi et al., 2010

<sup>x</sup> Agblevor et al., 1992

<sup>w</sup> Stolarski et al., 2011

**Table 7. Height, age and calculated above ground dry biomass of *Pterocarya stenoptera* as sampled from various locations. All plants produced from the same seed lot at the USDA Plant Introduction Station at Ames, IA.**

Location	Height (m)	mean height (m)	Age	Biomass <sup>z</sup> (kg)	mean biomass (kg)
Illinois, Glencoe	4.8		17	94	
Illinois, Urbana	11.0	11.5	17	1341	1258
	12.0		17	1174	
Iowa	10.5	11.0	17	387	431
	11.5		17	476	
Kansas	8.1	7.2	17	69	61
	6.3		17	54	
Missouri	12.0	12.0	17	907	868
	12.0		17	829	
Nebraska	9.4	8.2	17	252	158
	7.0		17	64	
South Dakota	7.4	7.1	17	133	125
	6.8		17	116	
Wisconsin	12.0		17	307	

<sup>z</sup> Dry biomass estimates based upon models by Lambert et al., 2005 and Ung et al., 2008.

**Table 8. Percentage of major chemical components of stems sourced from the energy farm and from 2<sup>nd</sup> year growth collected from various locations in the Midwest.** All plants utilized in this study originated from common offspring that were seed propagated from a 1985 Holden Arboretum (Kirtland, OH) seed collection trip to Kwangnung Forest Research Station, Kyong Gi Do, Namyeongju Gun, South Korea, along a stream in a flood plain.

	<b>n</b>	<b>cellulose</b>	<b>hemicellulose</b>	<b>lignin</b>	<b>ash</b>	<b>extractibles</b>	<b>acetyl</b>
Energy farm	24	25.2 ± 1.4	17.9 ± 0.54	22.1 ± 0.85	1.7 ± 0.25	14.4 ± 1.15	3.3 ± 0.19
Var. Locations	8	23.4 ± 1.89	15.7 ± 1.04	25.3 ± 0.97	2.7 ± 0.71	14.9 ± 1.53	3.0 ± 0.24

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# Appendix

## Coppice regeneration

### Literature review

Short rotation woody crops (SRWC) utilize fast growing coppice regrowth planted at a high density and mechanically harvested. To that end, species used in SRWC must possess the characteristic of producing many fast growing shoots from a single stump cut close to the ground and have the ability to tolerate repeated cutting cycles ranging from 2 to 5 years (Sennerby-Forsse et al., 1992). In coppice regrowth, shoots or sprouts are supported by the original root system and replace the original trunk usually sprouting from the collar. The collar is generally defined as the point on the main stem where the root and shoot systems come together (Sutton and Tinus, 1983). Collar formation in angiosperms and some gymnosperms initially forms above the cotyledonary node where meristematic tissue develops into buds with direct connection to the vascular cylinder (Del Tredici, 2001). The cotyledonary buds then give rise to accessory buds at the cotyledonary node (Del Tredici, 2001). Bud initiation that spreads up the stem plus the axillary buds from the first season of growth eventually forms an identifiable collar with separate bud clusters formed into an almost continuous band at the base of the trunk (Del Tredici, 2001).

In mature trees, the collar is located at or just below ground level and is composed of numerous suppressed buds that protrude slightly from the trunk (Del Tredici, 2001). Buds located on the collar grow slowly and can persist for years growing in length only enough to keep pace with the increasing diameter of the collar (Sakai et al., 1995; Del Tredici, 2001). The density of suppressed buds decreases further up the trunk, with buds located higher up known as epicormic buds. These two types of buds are identical in structure but epicormic buds form new branches when conditions are favorable (Kozlowski, 1971; Zimmerman and Brown, 1971). Viability of collar buds varies by species with some species having buds that remain viable for many years with other species losing bud viability relatively early (Del Tredici, 2001).

In most cases, angiosperm trees respond to cutting of the trunk with sprouting of suppressed buds below the point of injury with buds closest to the injury sprouting fastest (Del Tredici, 2001). Thus, in a coppicing system, the height at which the plant stem is cut can affect the regeneration of the plant. When a plant does regrow from coppicing, 75-90% of the new initial sprouts will die within five to ten years, with the most vigorous remaining (Johnson, 1975; Wendel, 1975). The majority of coppice regrowth sprouts die from heart rot due to the decomposition of the surrounding stump material. In order to avoid this, cutting the stem as close to the ground as possible is recommended (Wendel, 1975).

## Materials and methods

In July of 2011, a study was conducted to determine how the number of buds remaining after cutting the lower portion of the main stem would affect coppice regrowth. To study the effect of cutting height on coppicing ability, four treatments were designed to reflect possible cutting heights upon harvest. The treatments consisted of first stripping all leaves from the plant and then cutting the main stem so that 0, 5, 10, or 15 buds remained with treatments having average heights of 4.6 ( $\pm 2.5$ ), 8.0 ( $\pm 1.7$ ), 11.9 ( $\pm 2.5$ ), and 15.3 ( $\pm 2.3$ ) cm, respectively. The study was a randomized complete block design with four bud number treatments, five replications and 7 subsamples for a total of 140 plants on one greenhouse bench.

Trees utilized in this study were planted in November 2010 and grown for 9 months from seed obtained from the USDA North Central Region Plant Introduction Station located in Ames Iowa (seed lot number PI596388). The trees were watered as needed and grown under high pressure sodium lights (1000W GE Lucalox bulb, Cleveland, OH.), positioned approximately 1.8m above tree canopy, providing approximately 250  $\mu\text{mol}$  PAR supplemental lighting. Trees were fertilized with Scotts Brand Peters Professional water soluble fertilizer having a composition of 20N-8.76P-16.6K with M-77 micronutrient system (The Scotts Company, Marysville, OH.). Fertilizer was applied weekly at a rate of 250 ppm N. Conserve SC (Dow AgroSciences, Indianapolis, IN) (2 ml/gallon) was applied once for a minor infestation of thrips. The greenhouse was maintained with a heating target of 23°C and a cooling target of 28°C. Thirty-three days following removal of all leaves and the main stem to one of four bud number treatments, the number of shoots and leaves was recorded as well as total leaf dry mass per plant. Dry mass was analyzed via Analysis of Variance (ANOVA) using R (R Core Team, 2012). After diagnostic plots showed the assumptions of the model were met, mean separation via a protected Fisher's Least Significant Difference test was conducted using the `LSD.test()` function in the `Agricolae` package in R (de Mendiburu, 2012). Shoot and leaf count data were analyzed via Analysis of Deviance in R. With no

indication of overdispersion, the model was fit to a Poisson distribution. Mean separation (for instances where the main effect was found significant) was conducted as a series of Tukey-adjusted contrasts through the multcomp package in R (Hothorn et al., 2008).

## Results

Analysis of variance indicated that dry mass was affected ( $\alpha = 0.001$ ) by the number of buds left remaining on the stem, with blocking being non-significant. Eighty-eight percent of the plants with 0 buds remaining did not show any regrowth. Due to the high rate of mortality, the 0 bud treatment was not included in the means separation test. Treatments with 5 and 10 buds had similar mean shoot dry masses of 3.74 and 3.71 g per plant (Table A.1). Plants with 15 buds remaining after coppicing had a mean dry shoot mass of 4.37 g, significantly higher than the other treatments, indicating a positive relationship between the number of buds and the drymass of regrowth.

For both shoot and leaf count, blocking was not significant, making blocking non-significant for all three measures (mass, shoot count, and leaf count), indicating homogeneous conditions, and care during duration of the study. The number of shoots regenerated from the three treatments was significant ( $\alpha = 0.001$ ); however, the number of leaves produced by treatments was not significant, suggesting that plants with fewer shoots had a greater density of canopy.

For shoot production, treatments of 15 and 10 buds were similar; however plants with 5 buds were significantly different from treatments of 10 and 15 buds at an alpha level of 0.001. Plants with 5 buds produced a mean number of 6.9 shoots while treatments 10 and 15 produced 9.6 and 10.3 shoots, respectively.

Analysis of both dry mass and shoot production, indicate that short-term shoot biomass production increases with the number of buds remaining after coppicing, although it appears that the increase is not linear as expected based on the significance grouping of treatments in both studies. Leaf production did not increase with number of remaining buds with treatments 5, 10, and 15 yielding leaf counts of 38, 42, and 40, respectively. Given that number of leaves did not significantly change with treatment, it could be possible that the differences in dry mass might not be detectable over extended periods of growth. If this study were to be repeated, a measure of leaf area as well as a leaf count would be useful in interpreting differences in plant response and potential for longer term growth rate.

This study demonstrated that coppice regeneration is possible with the treatment of 15 remaining buds producing the best results. Given that treatment 0 experienced an 88% failure rate, it is reasonable to assume that young Chinese wingnut (< 1 year) have a limited number of epicormic buds. The results reported here are preliminary, a natural extension of this would be the development of a

study that tested coppicing ability on field grown trees that ranged in age from two to four years. A study designed to test the long term coppicing ability of the species could also be useful.

**Table A.1 Mean dry mass of *Pterocarya stenoptera* regrowth as a result of coppicing treatment (July, 2011).** *Pterocarya stenoptera* grown in 1 gallon containers (16.5 x 17.7 cm) under greenhouse conditions with supplemental lighting. Study duration 33 days.

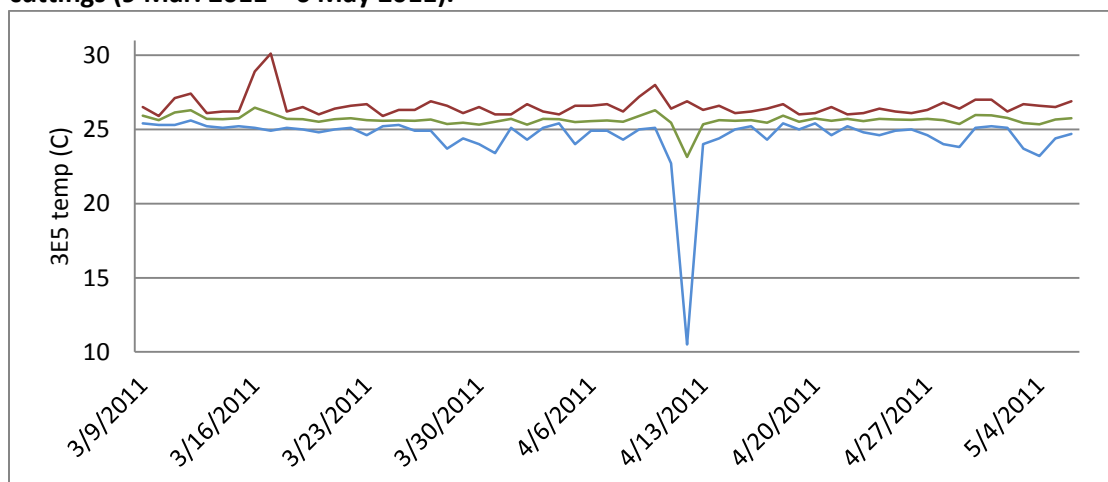
Treatment <sup>z</sup>	Dry mass(g)	n	Std. err	LCI	UCI
95% CI					
15	4.37 a <sup>y</sup>	33	0.171	4.04	4.71
10	3.74 b	34	0.222	3.30	4.19
5	3.71 b	34	0.122	3.47	3.95

<sup>z</sup>Number of buds remaining after initial pruning

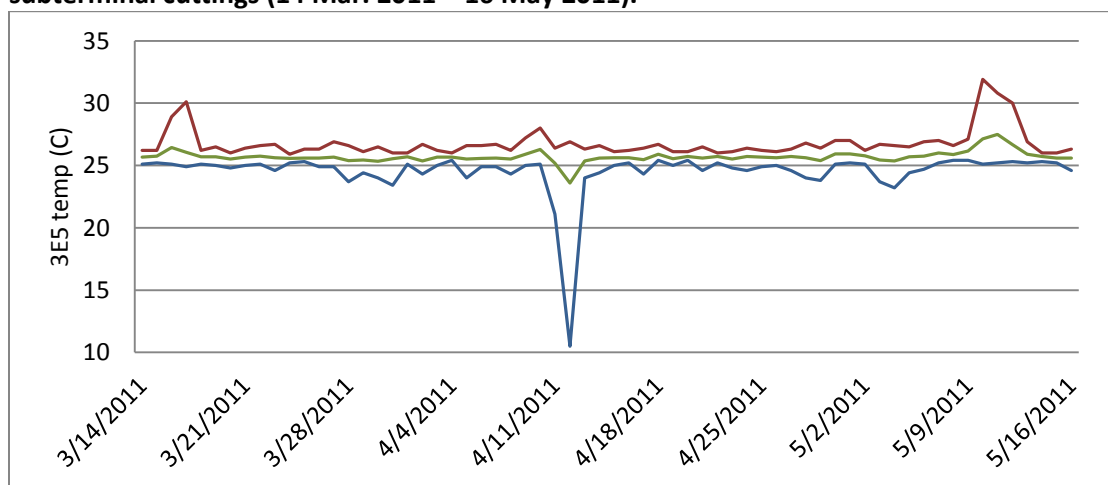
<sup>y</sup>Any two means in a column not followed by the same letter are significantly different at  $P \leq 0.05$



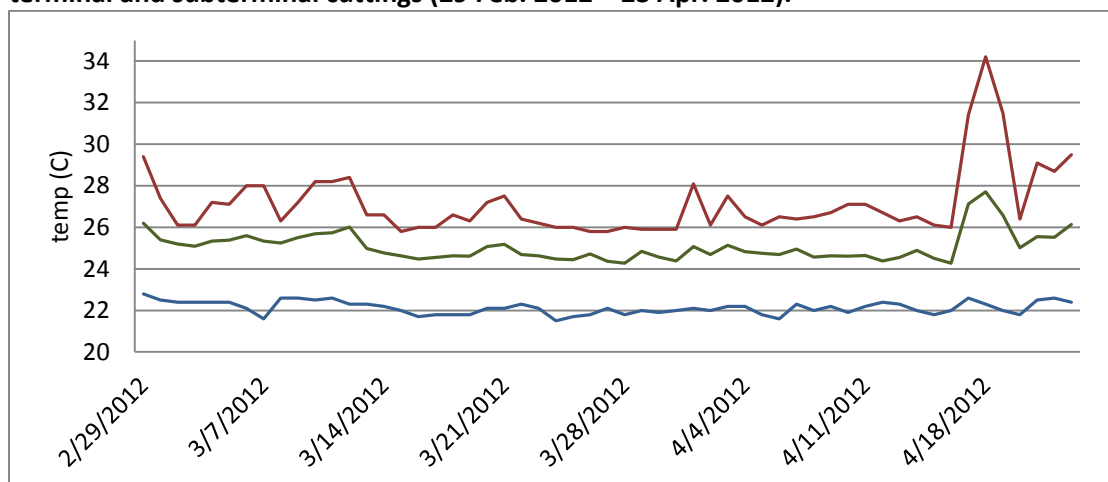
**Table A.2 High, low, and average temperatures during the first trial of hardwood terminal cuttings (9 Mar. 2011 – 6 May 2011).**



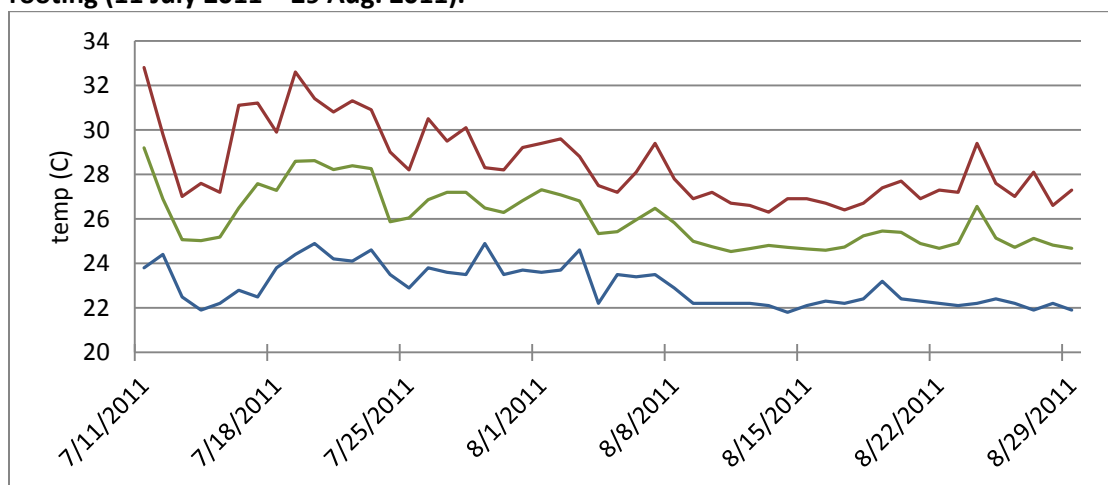
**Table A.3 High, low, and average temperatures during the first trial of hardwood subterminal cuttings (14 Mar. 2011 – 16 May 2011).**



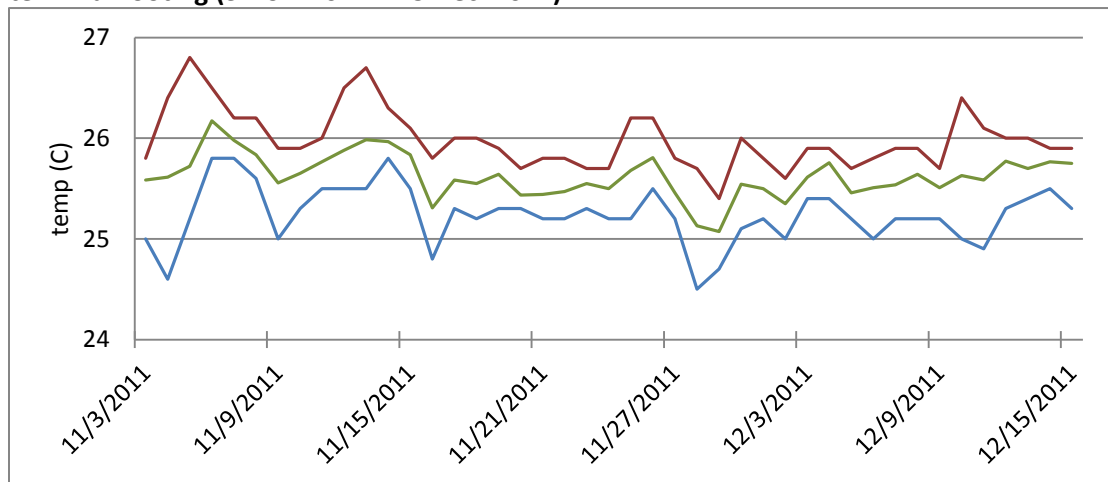
**Table A.4 High, low, and average temperatures during the second trial of hardwood terminal and subterminal cuttings (29 Feb. 2012 – 23 Apr. 2012).**



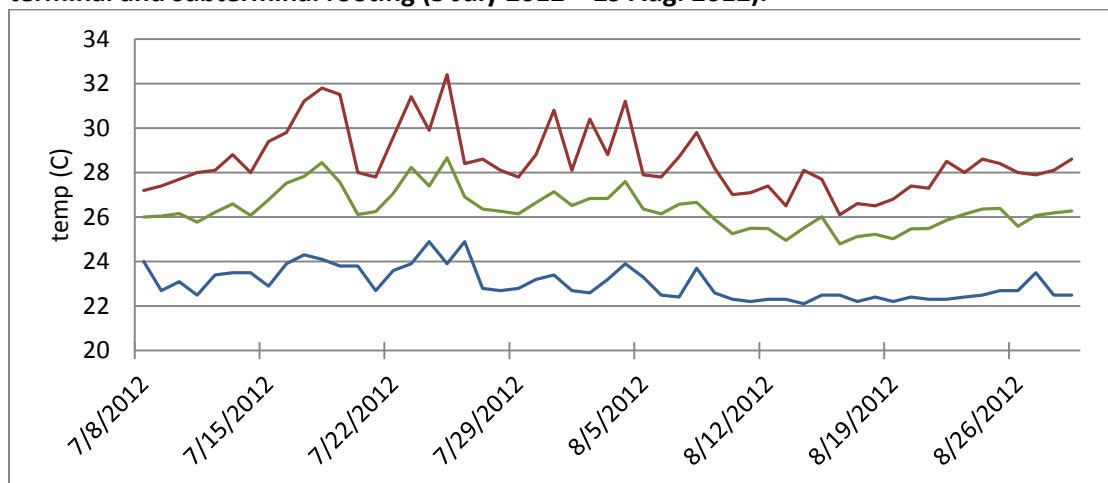
**Table A.5 High, low, and average daily temperatures during first trial of semi-hardwood subterminal rooting (11 July 2011 – 29 Aug. 2011).**



**Table A.6 High, low, and average daily temperatures during first trial of semi-hardwood terminal rooting (3 Nov. 2011 – 15 Dec. 2011).**

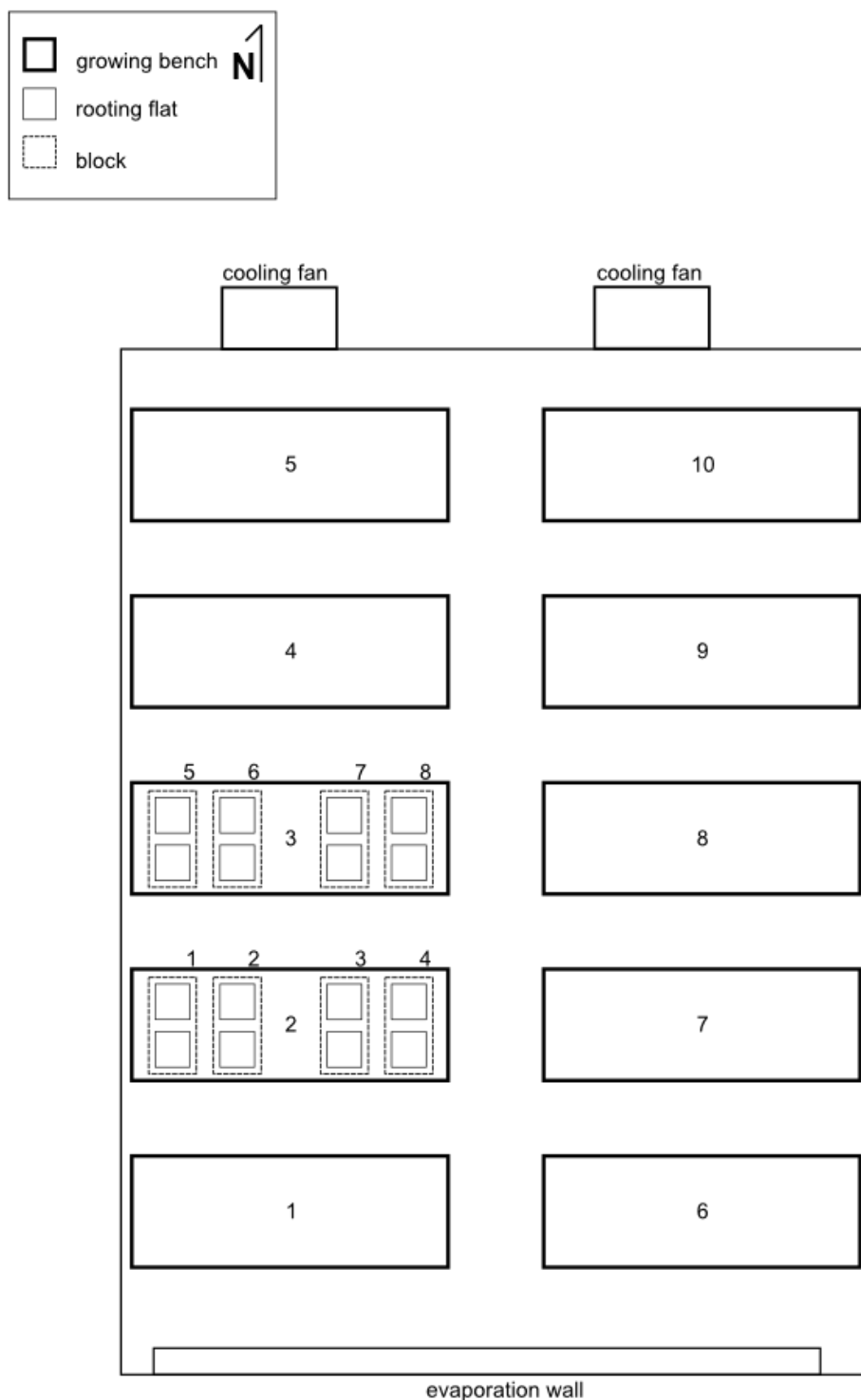


**Table A.7 High, low, and average daily temperatures during second trial of semi-hardwood terminal and subterminal rooting (5 July 2012 – 29 Aug. 2012).**

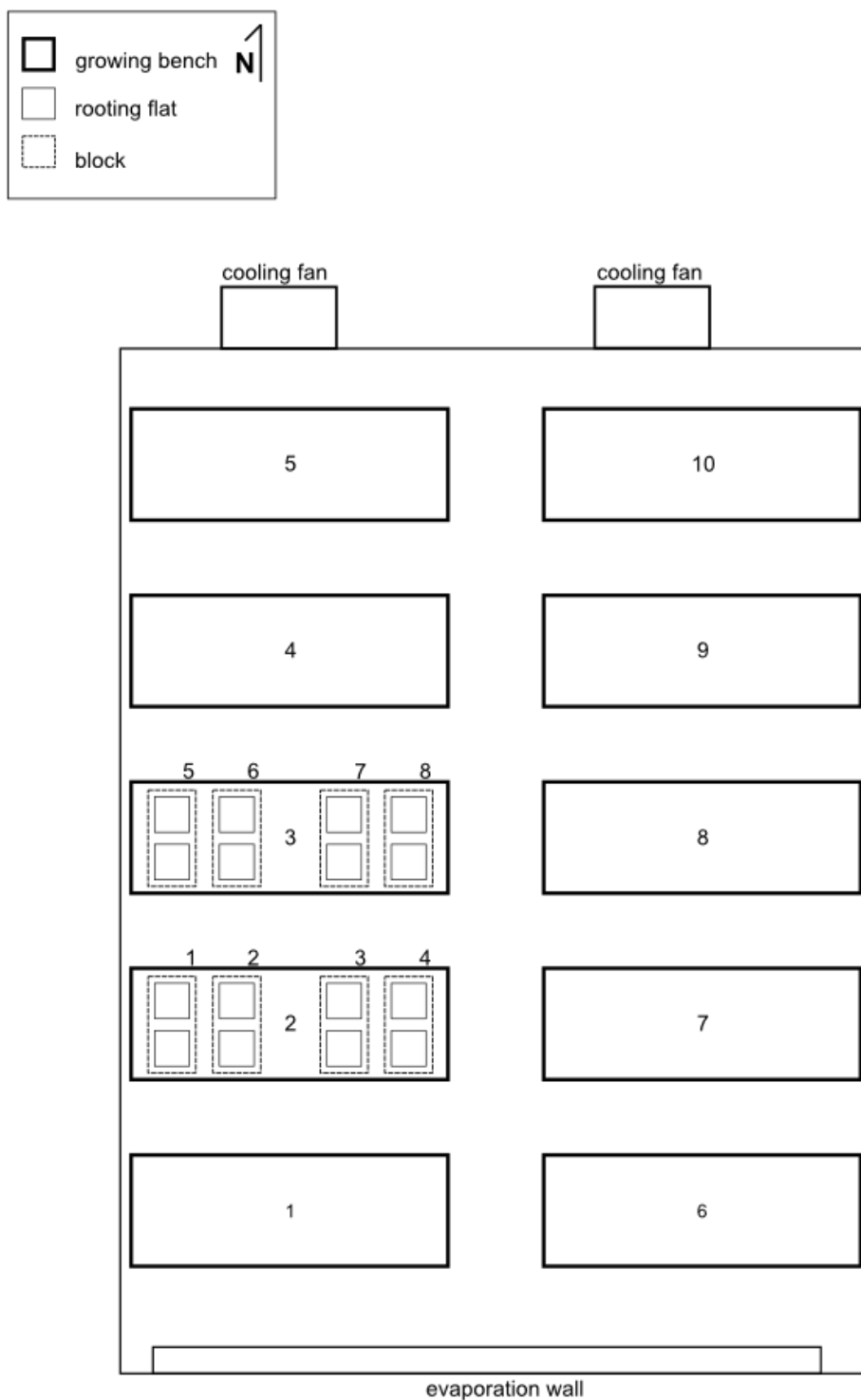


**Table A.8 Growing flat and block location for of second trial hardwood terminal cuttings of *Pterocarya stenoptera* treated with 0 to 5000 ppm IBA applied with liquid dip and wounded and non-wounded treatment.**

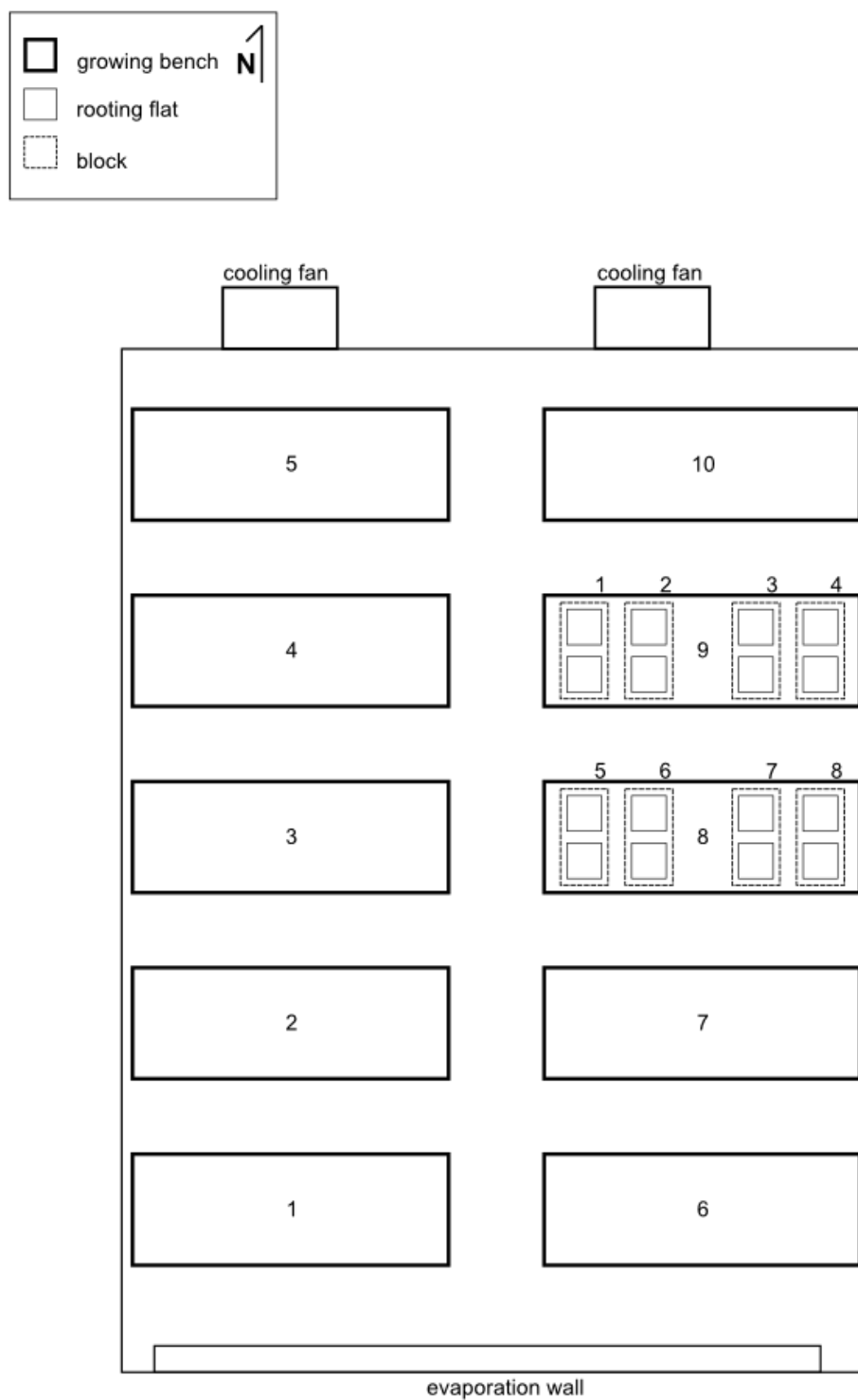
Cuttings wounded by removal of a single 2 to 3cm long sliver of bark from the basal end of the cutting exposing the cambium consists of removing a (February, 2012). Study duration 54 days.



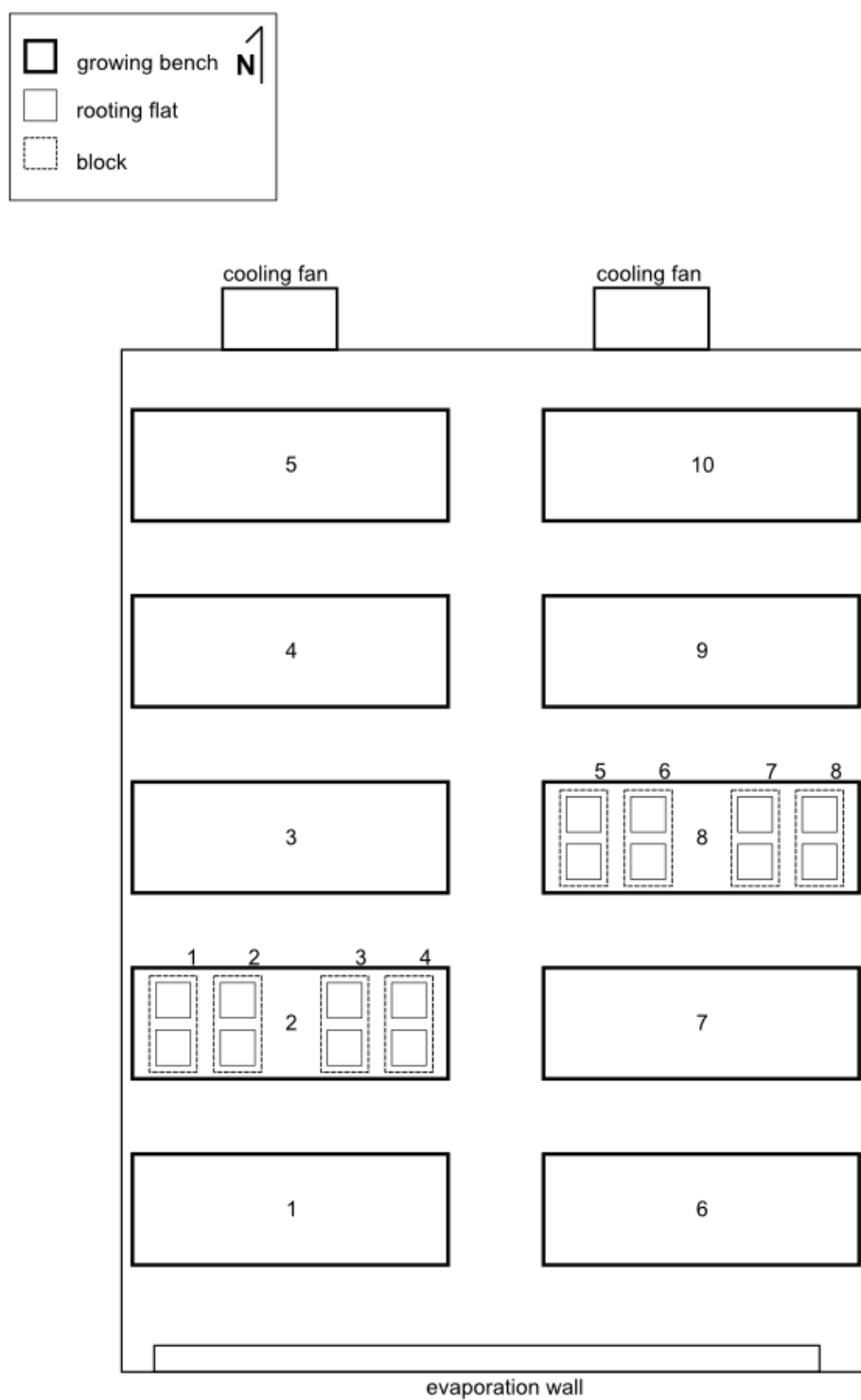
**Table A.9 Rooting flat and block location for second trial semi-hardwood terminal cuttings of *Pterocarya stenoptera* treated with 0 to 8000 ppm of IBA applied with liquid or talc-based carriers (July, 2012).** Study duration 55 days.



**Table A.10 Rooting flat and block location for second trial semi-hardwood sub-terminal cuttings of *Pterocarya stenoptera* treated with 0 to 8000 ppm of IBA applied with liquid or talc-based carriers (July, 2012).** Study duration 55 days.



**Table A.11 Rooting flat and block location of first trial semi-hardwood terminal cuttings of *Pterocarya stenoptera* treated with 0 to 8000 ppm of IBA applied with liquid or talc-based carriers (November, 2011). Study duration 43 days.**



**Table A.12 Rooting flat and block location of first trial semi-hardwood sub-terminal cuttings of *Pterocarya stenoptera* treated with 0 to 8000 ppm of IBA applied with liquid or talc-based carriers (July, 2011). Study duration 50 days.**

